Table S1

| Name                     | Sequence (5’-3’ )                     |
|--------------------------|---------------------------------------|
| SHANK2_Ex1F              | agacacccgccacctctc                   |
| SHANK2_Ex1R              | ggcaatatttaggaggaactgg                |
| SHANK2_Ex2F              | gaactcaacgtctccccctctg               |
| SHANK2_Ex2R              | gctctcgtcaatctttcttg                 |
| SHANK2_Ex3F              | catctctctgtcttgacatca                |
| SHANK2_Ex3R              | ggaatctcattcccagttga                 |
| SHANK2_Ex4F              | ggaagtgcagttacacccagga              |
| SHANK2_Ex4R              | gatgaaggccaggaagtaa                 |
| SHANK2_Ex5F              | cagactctccccagagcacg                |
| SHANK2_Ex5R              | tccatagtccctaccccgga                 |
| SHANK2_Ex6F              | cacctgcaaggtctattgt                 |
| SHANK2_Ex6R              | ggaacatattcaggtctcaga               |
| SHANK2_Ex7F              | ctggacactagccacctgaat                |
| SHANK2_Ex7R              | cacaagcaccacccacat                  |
| SHANK2_Ex8F              | ctccacagtcacagacagc                 |
| SHANK2_Ex8R              | gtgggatcagctggagagag                |
| SHANK2_Ex9_10F           | caccgctttctcccctcat                 |
| SHANK2_Ex9_10R           | ctgggctaggggatgtggtg                 |
| SHANK2_Ex12F             | ttctagttgccatctgcttg                |
| SHANK2_Ex12R             | gcactgatgacccgaagtaa                |
| SHANK2_Ex13F             | cttcaatgtctggttgttcta               |
| SHANK2_Ex13R             | gatgttcaatgtgtctccta                |
| SHANK2_Ex14F             | cttggtgtgcttggaaatgc                |
| SHANK2_Ex14R             | attgcaaaagatggcctgttc               |
| SHANK2_Ex15-1F           | gctggtgcttggttctcga                 |
| SHANK2_Ex15-1R           | caggctggatgtcttcacc                 |
| SHANK2_Ex15-2F           | aacctccgcaacaaagagagagg             |
| SHANK2_Ex15-2R           | ctgtgatggtggatgacata                |
| SHANK2_Ex15-3F           | gctgaatcctcagctcgaag                |
| SHANK2_Ex15-3R           | aaatacaccgccttctcct                 |
| SHANK2_Ex15-4F           | aagccagaggtggagatga                 |
| SHANK2_Ex15-4R           | tgtcaggtgtccacatct                 |
| SHANK2_Ex15-5F           | gactctggatcagggaggt                 |
| SHANK2_Ex15-5R           | cccacacaggttttaagat                 |
| SHANK2_Ex16-1F           | catggcatactccagttca                 |
| SHANK2_Ex16-1R           | atcggccccatctgtttag                 |
| SHANK2_Ex16-2F           | tggggctatctgtgctgta                 |
| SHANK2_Ex16-2R           | gtctgaagccacgcacctgt                |

Table S1. Primers used for SHANK2 sequencing in SCZ individuals and controls. PCR primers were designed using the Primer 3 software (v. 0.4.0) to amplify the exons of SHANK2 and the flanking exon-intron boundaries.
| Mutation | Mutation Taster | Poly Phen 2 | Poly Phen 2 | SIFT Best | SIFT All |
|----------|----------------|-------------|-------------|-----------|---------|
|          | Polymorphism   | Hum Div     | HumVar      | BLAST hit | BLAST hits |
| T438M    | Polymorphism   | Benign      | Benign      | Tolerated | Affected protein function |
|          | 0.9999        | 0.077       | 0.028       | 0.06      | 0.01    |
| G488V    | Disease causing | Benign      | Benign      | Tolerated | Affected protein function |
|          | 0.9907        | 0.011       | 0.021       | 0.21      | 0.03    |
| S610Y    | Disease causing | Possibly damaging | Possibly damaging | Affected protein function | Affected protein function |
|          | 0.9954        | 0.906       | 0.828       | 0.00      | 0.00    |
| N690S    | Polymorphism   | Benign      | Benign      | Tolerated | Tolerated |
|          | 0.9819        | 0.001       | 0.009       | 0.12      | 0.21    |
| R958S    | Disease causing | Probably damaging | Possibly damaging | Tolerated | Affected protein function |
|          | 0.7783        | 0.955       | 0.705       | 0.03      | 0.05    |
| P1119T   | Disease causing | Probably damaging | Probably damaging | Affected protein function | Affected protein function |
|          | 0.9999        | 0.999       | 0.997       | 0.01      | 0.00    |
| P1144L   | Disease causing | Probably damaging | Probably damaging | Tolerated | Affected protein function |
|          | 0.9998        | 0.999       | 0.995       | 0.07      | 0.00    |
| V1608I   | Polymorphism   | Benign      | Benign      | Tolerated | Tolerated |
|          | 0.8409        | 0.013       | 0.053       | 0.50      | 0.55    |
| L1646M   | Disease causing | Possibly damaging | Possibly damaging | Affected protein function | Tolerated |
|          | 0.9944        | 0.370       | 0.380       | 0.03      | 0.06    |
| A1731S   | Disease causing | Benign      | Benign      | Tolerated | Benign |
|          | 0.6632        | 0.000       | 0.003       | 0.65      | 0.47    |

**Table S2.** Ten missense SHANK2 variants found in SCZ patients and not present in our controls. The given prediction values for MutationTaster, PolyPhen2 and SIFT are from December 2011. These values were used for the initial prioritizing of variants for functional studies. Annotations are done according to sequence NP_036441.2.
Table S3

Table with all identified SHANK2 variants in SCZ patients. We used different \textit{in silico} prediction tools for characterization of the variants: MutationTaster\textsuperscript{,1} Provean\textsuperscript{,2} wANNOVAR\textsuperscript{3} and CONDEL\textsuperscript{4}. The given values are from March 2014. The variants were checked for presence in the 1000 Genome project\textsuperscript{5} and Exome Variant Server\textsuperscript{6} databases.
| Mutation   | Controls (n=659) | SCZ (n=481) |
|------------|------------------|-------------|
| p.T438M    | -                | 1           |
| p.G488V    | -                | 1           |
| p.S610Y    | -                | 1           |
| p.N690S    | -                | 1           |
| p.R958S    | -                | 1           |
| p.P1119T   | -                | 1           |
| p.P1144L   | -                | 1           |
| p.V1608I   | -                | 1           |
| p.L1646M   | -                | 1           |
| p.A1731S   | -                | 4           |
| p.R569H    | 16               | 4           |
| p.R818H    | 4                | 9           |
| p.Y967C    | 16               | 14          |
| p.I1668T   | 2                | 6           |
| p.M1717I   | 2                | 5           |
| p.T410M    | 1                | -           |
| p.R415W    | 1                | -           |
| p.D432N    | 1                | -           |
| p.E514G    | 1                | -           |
| p.S557N    | 2                | -           |
| p.K780Q    | 4                | -           |
| p.T796N    | 1                | -           |
| p.P801T    | 1                | -           |
| p.T825M    | 1                | -           |
| p.A1429S   | 2                | -           |
| p.P1456T   | 1                | -           |
| p.P1586L   | 1                | -           |
| p.D1696N   | 1                | -           |

**Total** 58 51

**Table S4.** SHANK2 missense variants identified in controls and SCZ patients. List of all missense variants identified in patients and/or controls. Color code, red: variants found only in patients; orange: variants identified in both patients and controls; green: variants found only in controls. Annotations are done according to sequence NP_036441.2. No enrichment of missense variants in patients was observed (Fisher’s exact test, 2-sided: $P = 0.310$).
| Position       | Amino Acid | Frequency SCZ (N=481) | Frequency Controls (N=659) |
|----------------|------------|-----------------------|----------------------------|
| G70666733A     | p.T410M    | 0                     | 1                          |
| G70666719A     | p.R415W    | 0                     | 1                          |
| C70666668T     | p.D432N    | 0                     | 1                          |
| G70666649A     | p.T438M    | 1                     | 0                          |
| C70666499A     | p.G488V    | 1                     | 0                          |
| T70653229C     | p.E514G    | 0                     | 1                          |
| C70644655T     | p.S557N    | 0                     | 2                          |
| G70544817T     | p.S610Y    | 1                     | 0                          |
| T70349029C     | p.N690S    | 1                     | 0                          |
| T70338541G     | p.K780Q    | 0                     | 4                          |
| G70338492T     | p.T796N    | 0                     | 1                          |
| G70338478T     | p.P801T    | 0                     | 1                          |
| C70336479T     | p.R818H    | 9                     | 4                          |
| G70336458A     | p.T825M    | 0                     | 1                          |
| G70333526T     | p.R958S    | 1                     | 0                          |
| G70333043T     | p.P1119T   | 1                     | 0                          |
| G70332967A     | p.P1144L   | 1                     | 0                          |
| A70332111G     | p.A1429S   | 0                     | 2                          |
| G70332032T     | p.P1456T   | 0                     | 1                          |
| G70331641A     | p.P1586L   | 0                     | 1                          |
| C70331576T     | p.V1608I   | 1                     | 0                          |
| G70331462T     | p.L1646M   | 1                     | 0                          |
| A70319533G     | p.I1664T   | 6                     | 2                          |
| C70319438T     | p.D1696N   | 0                     | 1                          |
| C70319373T     | p.M1717I   | 5                     | 2                          |
| C70319333A     | p.A1731S   | 4                     | 0                          |

**Total** 33 26

**Table S5.** Rare SHANK2 missense variants found in 481 SCZ patients and in 659 controls. Variants with MAF<1% in controls were considered for the mutation burden analysis in Sanger-sequenced cohorts. Annotations are done according to sequence NP_036441.2.
Table S6

Clinical information for individuals that harbor SCZ specific SHANK2 variants in our cohort.
Table S7

A. SHANK2 protein conservation in mutated positions - multiple sequence alignments. A. Multiple sequence alignments presenting -/+ 5 amino acids around mutated positions in seven different species. Affected positions are marked in yellow. Variants selected for functional analysis are marked in blue. Generated by MUSCLE version 3.6 (using option: -maxiters 2). B. Genes and proteins in different species used for the alignment. Genes were identified as putative homologs of one another during the construction of HomoloGene.

| Variant      | T438M | G488V | S610Y | N690S | R958S |
|--------------|-------|-------|-------|-------|-------|
| H. sapiens   | WAVCS | ATSHR | GAGED | G      | HYTVGS |
|              |       |       | YDSD  | N     | YDSD  |
|              |       |       | LIEVN | N     | ENVK  |
|              |       |       | RRELD | R     | YSLDS |
| R. norvegicus| WAVCS | ATSHR | GAGED | G      | HYTVGS |
|              |       |       | YDSD  | N     | YDSD  |
|              |       |       | LIEVN | N     | ENVK  |
|              |       |       | RRELD | R     | YSLDS |
| M. musculus  | WAVCS | ATSHR | GAGED | G      | HYTVGS |
|              |       |       | YDSD  | N     | YDSD  |
|              |       |       | LIEVN | N     | ENVK  |
|              |       |       | RRELD | R     | YSLDS |
| B. taurus    | WAVCS | ATSHR | GAGED | G      | HYTVGS |
|              |       |       | YDSD  | N     | YDSD  |
|              |       |       | LIEVN | N     | ENVK  |
|              |       |       | RRELD | R     | YSLDS |
| G. gallus    | WSASS | A      | ---   |       |       |
|              |       |       |       |       |       |
| D. rerio     | RSPSP | A      | ---   |       |       |
|              |       |       |       |       |       |

| Variant      | P1119T | P1144L | V1608I | L1646M | A1731S |
|--------------|--------|--------|--------|--------|--------|
| H. sapiens   | LGPA   | PRTRPS | EQLSS  | P      | MPSAT  |
|              |        |        | P      |        | KLWGD  |
|              |        |        | D      |        | VTEIKS |
|              |        |        |        |        | KPECGE |
|              |        |        |        |        | DSPMG  |
|              |        |        |        |        | AAAAA  |
| R. norvegicus| LGPA   | RMQPS  | EQPLL  | P      | TPQGA  |
|              |        |        | KLWGD  | E      | MPVKS  |
|              |        |        | KLWGD  | V      | P    |
|              |        |        | KLWGD  | V      | P    |
| M. musculus  | LGPA   | RMQAS  | EQPLL  | P      | TPQGA  |
|              |        |        | KLWGD  | E      | MPVKS  |
|              |        |        | KLWGD  | V      | P    |
|              |        |        | KLWGD  | V      | P    |
| C. lupus     | LGPA   | RVHSS  | EPLSL  | P      | TPQGAV |
|              |        |        | KLWGD  | E      | MPVKS  |
|              |        |        | KLWGD  | V      | P    |
|              |        |        | KLWGD  | V      | P    |
| B. taurus    | LGPA   | RARPS  | EPLSS  | P      | ---    |
|              |        |        | KLWGD  | E      | MPVKS  |
|              |        |        | KLWGD  | V      | P    |
|              |        |        | KLWGD  | V      | P    |
| G. gallus    | LTPPT  | RMQGS  | RQLMS  | P      | SPIPA  |
|              |        |        | KLWGD  | E      | MPVKS  |
|              |        |        | KLWGD  | V      | P    |
|              |        |        | KLWGD  | V      | P    |
| D. rerio     | VPTPT  | RLRHS  | RRLMA  | P      | ---    |
|              |        |        | KLWGD  | E      | MPVKS  |
|              |        |        | KLWGD  | V      | P    |
|              |        |        | KLWGD  | V      | P    |

B. Organism | Gene | Protein |
-------------|------|---------|
H. sapiens  | SHANK2 | NP_036441.2 |
C. lupus    | SHANK2 | NP_540798.3 |
B. taurus   | SHANK2 | XP_002699471.1 |
M. musculus | Shank2 | NP_001106844.2 |
R. norvegicus | Shank2 | NP_958738.1 |
G. gallus   | SHANK2 | XP_426415.3 |
D. rerio    | LOC567595 | NP_001121819.1 |
### Table S8

| Position       | AA      | Found in | Effect on synaptic density | MutationTaster | PolyPhen2 Div | Sift Best Blast Hits |
|----------------|---------|----------|----------------------------|----------------|---------------|----------------------|
| G70544817T     | p.S610Y | SCZ      | yes                        | disease causing | probably damaging | affect protein function |
| G70333526T     | p.R958S | SCZ      | yes                        | disease causing | probably damaging | affect protein function |
| G70333043T     | p.P1119T| SCZ      | yes                        | disease causing | probably damaging | affect protein function |
| C70319333A     | p.A1731S| SCZ      | yes                        | disease causing | benign         | tolerated             |
| C70644619T     | p.R569H | SCZ, ASD, controls | yes                        | disease causing | probably damaging | affect protein function |
| T70338541G     | p.K780Q | ASD, controls | no                         | disease causing | probably damaging | affect protein function |
| C70336468T     | p.A822T | controls | no                         | disease causing | possibly damaging | affect protein function |
| C70336465T     | p.V823M | controls | no                         | disease causing | probably damaging | affect protein function |
| T70333498C     | p.Y967C | SCZ, ASD, controls | no                        | disease causing | probably damaging | affect protein function |
| G70332530A     | p.R1290W| controls | no                         | polymorphism    | probably damaging | affect protein function |
| G70331641A     | p.P1586L| ASD, controls | no                         | disease causing | probably damaging | affect protein function |
| C70644655T     | p.S557N | ASD, controls | yes                        | disease causing | probably damaging | affect protein function |
| A70507751G     | p.L629F | controls | no                         | disease causing | probably damaging | affect protein function |
| C70348949A     | p.V717F | ASD      | yes                        | disease causing | probably damaging | affect protein function |
| Variation       | Allele | Disease | Prediction | Function       |
|-----------------|--------|---------|------------|----------------|
| C70348913T      | p.A729T| ASD     | yes        | disease causing|
| C70336479T      | p.R818H| SCZ, ASD, controls | yes | disease causing |
|                 |        |         |            | probably damaging |
| C70332890T      | p.G1170R| ASD, controls | yes | polymorphism |
|                 |        |         |            | probably damaging |
|                 |        |         |            | tolerated |
| T70332475C      | p.Q1308R| controls | no | disease causing |
|                 |        |         |            | benign |
|                 |        |         |            | tolerated |
| A70319359G      | p.L1722F| ASD     | yes        | disease causing |
|                 |        |         |            | possibly damaging |
|                 |        |         |            | affect protein function |
| C70331795T      | p.D1535N| ASD     | yes        | disease causing |
|                 |        |         |            | probably damaging |
|                 |        |         |            | affect protein function |

Table S8. Comparison of functional data to in silico predictions for 20 experimentally analyzed missense SHANK2 variants. Functional data comes from our study and Leblond et al., 2012. All annotations are done according to hg19 and protein sequence NP_036441.2. The functional analysis was performed by quantifying synaptic density. Prediction values for MutationTaster, PolyPhen2 and SIFT (September 2014) are given. Variants with disagreement between functional data and predictions are colored in blue.
Fig S1

(a) Number of Branch or Terminal Points
(b) Number of Processes/Cell
(c) Average Length of Processes/Cell
(d) Total Cube Length per Cell
(e) Number of Processes/Cell by Order
(f) Average Length of Processes/Cell by Order
(g) Shell Analysis WT/610Y
(h) Shell Analysis WT/R9385
Figure S1. Quantification of the dendritic arbor complexity and the overall neuronal morphology. The analysis was performed on 2D epifluorescent pictures using the Bonfire software. (a) Total number of terminal points per cell. (b) Total number of processes per cell. (c) Average length of processes per cell. (d) Total cable length per cell. (e) Number of processes per cell by order according to RIT labeling scheme. (f) Average length of processes per cell by order according to RIT labeling scheme. (g), (h), (i) and (j) Sholl analysis for all 4 tested variants in comparison with the wild type. RIT labeling scheme stands for “roots, intermediate, terminal” convention, in which any neurite originating in the soma is labeled root segment (R), any neurite with no daughter neurites is labeled as terminal segment (T), and any neurite that is not fitting to those categories is labeled as intermediate segment (I).
Figure S2. Analysis of spine density in primary hippocampal neurons over-expressing SHANK2-SH3 variants. Overexpression of either mCherry-SHANK2-SH3 wild type or any of the SCZ mutants showed no effect on spine density in primary hippocampal neurons; (n = 3 experiments, 18 neurons for each condition).
Figure S3. Analysis of the localization of SHANK2 schizophrenia variants to the actin stress fibers and focal adhesions. (a) SHANK2E mutants and wild type with YFP tag as N-terminal fusions were co-transfected together with RFP-actin into COS-7 cells. Live cell TIRF images are presented. YFP-SHANK2E formed aggregates around the actin fiber tips but did not co-localize with them. All variants tested showed localization similar to the wild type. Scale bar - 20 µm (main panels); 5 µm (insets). (b) YFP-SHANK2-SH3 mutants and wild type were co-transfected together with RFP-actin into COS-7 cells. Live cell TIRF images are presented. YFP-SHANK2-SH3 wild type formed intracellular clusters of different sizes. In some cells, small numbers co-localize with actin fiber tips but most are randomly distributed into the cell. All variants tested showed localization similar to the wild type. Scale bar - 20 µm (main panels); 5 µm (insets).
Clinical reports for patients with A1731S Variants*

Patient 1 (male, 28 years at interview; Schizophrenia, paranoid Subtype 295.30)

Socio-Demographics and Family History

The patient was born in the early seventies and grew up with his parents and older siblings. The father had a managerial position and the mother initially worked in a non-professional position and then as a house wife. All siblings have academic careers. The patient’s paternal grandmother was reported to have experienced psychosis with paranoia. The patient was delivered by forceps. His early childhood development was normal. He performed well in school until early adolescence, when he experienced his first failure in school examinations and his performance remained very poor thereafter. His parents described him as a “late developer”, and his teachers described him as being “mentally absent” in class. In late adolescence he began to abuse various drugs (e. g. Hashish, LSD, Mescaline, and Narcotics) on a regular basis and left school as a result.

Anamnesis

Disease onset was insidious. He first developed psychotic symptoms (voices) in late adolescence. The patient was first admitted to a psychiatric hospital at the age of 19 with severe sleep problems (initial and middle insomnia) and auditory hallucinations. He reported hearing the voices of both unknown and known (e. g. parents) persons. The voices were commenting and talking about him in an abusive, accusatory, and persecutory manner. He reported that initially, the voices had only occurred after he had taken drugs, but had then become persistent. He reported the feeling that people passing by on the street were talking about him and looking at him. He reported believing himself to be a superman and God, and he displayed socially inadequate behavior (e.g. getting undressed in public). He had no insight into his mental health problems. Mental state examination revealed widespread persecutory delusions, religious delusions, delusions of influence, and delusions of grandeur, in addition to the auditory hallucinations described above. He also displayed ego disturbances in the form of thought broadcast, thought insertion, thought withdrawal, and feelings of derealisation and depersonalization. He showed increased sociability. His affect alternated between flattened and foolish, latent aggressive, and provocative. He was agitated, had difficulties in sleeping and concentrating and displayed formal thought disorder (loosening of associations). During the hospital admission, he occasionally became despairing and suicidal. He was diagnosed with hebephrenic schizophrenia and treated with Benperidol, Levemepromazin, Flupentixol, and Biperiden.

The patient had several more hospital admissions over the following seven years, two of which lasted for a period of 2 years. He was treated with various psychotropic medications (Promethazin, Haloperidol, Bromperidol, Chlorprothixen, and Lorazepam). For six years he received maximum dose Clozapine (300mg/day). He also received treatment for depressive symptoms and underwent tranquilizer withdrawal. During the hospital admission at the age of 28 years he reported feeling depressed, hopeless, and internally tense. Mental state examination revealed delusions of influence and auditory hallucinations. He displayed almost constant facial grimacing, particularly in the ocular area. He was treated with Perazine, Pimozide, and Promethazine. By the age of 28 years, he had moved out of his parents’ home, and had commenced an apprenticeship tailored for individuals with chronic schizophrenia, which he found too demanding.

Structured Interview at age 28

A structured life-time interview was conducted at the age of 28 years when the patient consented to participate in genetic studies. During this interview the following additional symptoms were elicited: elevated mood, increased self-esteem, excessive activity, distractibility, reduced need for sleep, agitated and slowed activity, loss of energy, parathymia, dysphoria, and loss of pleasure. At that time point the disease course was chronic with deterioration from the premorbid level of functioning.

Clinical diagnoses assigned over disease course

Age 19: Hebephrenia
Age 25: Depressive syndrome with known schizophrenic psychosis
Age 28: Endogenous schizophrenic psychosis (ICD-9: 295.3), chronic paranoid-hallucinatory psychosis (schizophrenic)
Interview diagnosis
Schizophrenia, paranoid subtype (DSM-IV: 295.30)

Patient 2 (female, 17 years at interview; Schizophrenia, disorganized Subtype 295.10)

Socio-Demographics and Family History
The patient was born in the eighties and grew up with her parents and two older siblings. The father worked in a manual public service position, and the mother was a housewife. Her paternal granduncle had experienced a paranoid psychosis and remained in a psychiatric hospital for several decades until his death. After graduating from school (Realschule**) she commenced an apprenticeship. Her past medical history included a visible, and probably autoimmune, skin disease.

Anamnesis
The patient reported that she had suffered a depressive episode at the age of 14 which remitted spontaneously. She was first hospitalized for psychiatric problems at the age of 17. During the preceding weeks, her teachers and classmates had considered her behavior "strange": she could not concentrate in class, talked to herself, and went outside while she was supposed to be in the class room.

In hospital, she presented with an unstable mood that switched between depressed, cheerful, irritable and parathymic. At times she was parathymic, irritable, and verbally aggressive. She reported that she felt wound-up inside and could not concentrate. She was mute at times, and displayed formal thought disorder (loosening of associations, tangentiality). She displayed mannerisms, especially with regard to speech. She reported auditory hallucinations (music and noises) and feeling of derealization. She had widespread delusions of influence. She displayed bizarre behaviors and ego disturbances in the form of thought withdrawal and feelings of derealization. She showed increased sociability and was agitated. Her treating psychiatrists described her personality as insecure and childlike. She was treated with Haloperidol and Levomepromazine. Following the development of Parkinson-like symptoms and akathisia, the medication was discontinued and she was commenced on Clozapine. On hospital discharge, affective symptoms and mannerisms were still evident.

Structured Interview at age 17
The clinical lifetime interview took place when the patient was first hospitalized at the age of 17. At this time-point, the patient had had 2 episodes of psychiatric disturbance. In the lifetime interview the following additional symptoms were elicited: elevated mood, excessive activity, distractibility, reduced need for sleep, pressured speech, thought racing, agitated activity, loss of energy, restricted affect, diminished libido, sleeping problems, poor appetite, and weight loss.

Clinical diagnoses assigned over disease course
Age 14: Major depressive episode, single episode
Age 17: Acute schizophrenic episode (ICD-10: F23.2), Differential Diagnosis: Disorganized Schizophrenia (ICD-10: F20.1)

Interview diagnosis
Schizophrenia, disorganized subtype (DSM-IV: 295.10)

Patient 3 (female, 41 years at interview; Schizophrenia, paranoid Subtype 295.30, Differential Diagnosis: organic delusional disorder 293.81)

Socio-Demographics and Family History
The patient was born in late seventies. At the time of study participation she had no contact with her family and stated only that she had two siblings and no family history of psychiatric problems. After graduating from school (Hauptschule**) at around the age of 15 years, she commenced an apprenticeship and worked for a decade in the same company. In her early thirties she stopped work because she felt bullied by her colleagues. She described herself as a loner and lived alone.

Anamnesis
The patient suffered meningitis in early childhood and subsequently developed absences and seizures with aura. Following brain surgery in her early thirties, the seizures ceased but she felt that her personality had
changed, i.e. she reported that she had increasingly withdrew from social contacts and become more serious. Prodromal schizophrenia symptoms had been evident during her apprenticeship. She was first hospitalized at the age of 34 after a suicide attempt, which was precipitated by feeling bullied at work. At the age of 36, she lost a substantial amount of weight and was hospitalized on the basis of a court decision for being an acute danger to herself. She reported that she did not have money for food because everybody wanted to take her money and eliminate her, and thus she could not trust anyone. These ideas were classified as persecutory delusions by the treating psychiatrists. She also had delusions of grandeur, i.e. she believed herself to be the daughter of a famous person. She was anxious and suspicious, and had difficulties with concentration and memory. She had no insight into her mental health problems, and was allocated a legal guardian. She was treated with Levetiracetam, Carbamazepine, and Risperidone. At the age of 40, she was rehospitalized due to another episode of severe weight loss. Again, this had occurred because she believed that she did not have money for food. She reported feeling blank inside and bored, but also that “something positive was buzzing in the air”. This was classified by the treating psychiatrists as a primary delusional perception. She presented with widespread systematized persecutory delusions, delusions of influence, delusions of grandeur, and tactile hallucinations. She displayed ego disturbances in form of thought insertion. She had concentration and memory difficulties, mild formal thought disorder (her speech lacked logical structure and was difficult to understand) and increased sociability. Her affect was alternately depressed, flattened, and parathymic. She sometimes smiled incongruently. She was treated with Risperidone.

Structured Interview at age 41

The life-time interview took place when the patient was hospitalized at the age of 41. The following additional symptom was elicited: dysphoria. Prior to this interview the patient had experienced at least 3 disease episodes. The disease course was chronic, and deterioration from the premorbid level of functioning was apparent.

Clinical diagnoses assigned over disease course

Age 37: Organic delusional (schizophreniform) disorder (ICD-10: F06.2)
Age 41: Schizophrenia, paranoid subtype (ICD-10: F20.0), Differential Diagnosis: Organic delusional disorder (ICD-10: F06.2)

Interview diagnosis

Schizophrenia, paranoid subtype (DSM-IV: 295.30), Differential Diagnosis: Organic delusional disorder (DSM-IV: 293.81)

Patient 4 (male, 37 years at interview; Schizophrenia, paranoid Subtype 295.30)

Socio-Demographics and Family History

The patient was born in the late sixties and grew up with his parents and older sibling. The patient described his father as a strict and dominant person who regularly beat up the patient’s mother. The father died in the nineties. The sibling reported diverse problems in coping with life. After graduating from school (Gymnasium**), the patient completed two terms at University and then worked as a plumber for several years until his thirties when he lost his driving license. He then moved back into his mother’s home and lived off his savings.

Anamnesis

His relatives reported that from late adolescence the patient had shown an increasing tendency towards being a loner. When he was first hospitalized at the age of 32, he already had developed a delusional system of political and religious over the course of several years. He was first. At that time he was living with his mother and they were experiencing increasing conflict. While in an acute, agitated state he had threatened to injure her physically. He reported that he could foresee things and believed he was Jesus (delusions of grandeur). He had tactile hallucinations and believed that they were a sign of a forthcoming negative event (delusional perception). The patient presented with complex persecutory delusions, delusions of influence, and delusions of grandeur (religious and political content), as well as auditory, visual, and tactile hallucinations. He displayed ego disturbances in the form of thought withdrawal and formal thought disorder (loosening of associations, thought blocking) as well as slowed and inhibited thinking. He had difficulties concentrating and was absent-minded. He was agitated, irritable, and aggressive as well as anxious and suspicious. He had no insight into his mental health problems. He was treated with Flupentixol and Biperiden. The symptoms showed only partial remission. The treating psychiatrists described him as a humorous and sympathetic young man. He
was hospitalized for a second time at the age of 34 after he had discontinued his medication and become increasingly aggressive at home. He reported that he had not slept much. He was suspicious, had delusions of persecution and grandeur, and thought disruption. He was treated with Haloperidol, Propranolol, and Biperiden. Six months later he was rehospitalized with formal thought disorder (loosening of associations) as well as slowed and inhibited thinking. He also was absent-minded. His affect was restricted, anxious, and aggressively tense. He had visual and auditory hallucinations, misjudged the identities of people around him (delusional misidentification), and displayed stereotypies. He was treated with Flupentixol, Biperiden, and Levomepromazine. Although the treating psychiatrists suggested that a legal guardian should be appointed, the patient and his family refused. At the age of 38 he was rehospitalized when he was discovered walking around naked out-doors.

**Structured Interview at age 38**

The life-time interview took place in the nineties when the patient was hospitalized at the age of 38. The following additional symptoms were elicited: bizarre behavior, increased sociability, increased self-esteem, elevated mood, reckless activity, distractability, dysphoria, loss of pleasure, slowed activity, loss of energy, poor concentration, diminished libido, excessive self-reproach, suicidal ideation, primary delusional perceptions, delusions of guilt, and cannabis abuse.

By this time-point the course of the disease was chronic, and deterioration from the premorbid level of functioning was apparent.

**Clinical diagnoses assigned over disease course**

- Age 32: Schizophrenic psychosis
- Age 34: Acute exacerbation of schizophrenic psychosis
- Age 38: Acute exacerbation of paranoid-hallucinatory psychosis

**Interview diagnosis**

Schizophrenia, paranoid subtype (DSM-IV: 295.30)

**Summary**

All patients showed an early, non-specific onset of psychiatric symptoms and a prodromal phase with an insidious onset prior to first hospitalization with schizophrenia. All patients displayed substantial ego disturbances (depersonalization), persecutory delusions, and auditory hallucinations.

* To ensure confidentiality, certain personal details with no impact on relevant disease phenotypes have been changed.

** Until recently, pupils in the German school system were sent after the 4th grade (i.e. around the age of 10 years) and on the basis of their school performance to one of three different types of secondary school. The most academically gifted pupils attended the Gymnasium, students with average performance attended the Realschule, and students with low performance attended the Hauptschule.
Cloning of different SHANK2 constructs

All PCR amplifications were performed using Phusion Hot Start II High Fidelity DNA Polymerase (Thermo Scientific) or Q5 High-Fidelity DNA Polymerase (New England Biolabs). Annealing temperatures for all primer pairs were calculated using the NEB online Tm Calculator. Only template specific parts of the primers were used for this calculation excluding restriction sites, Kozak sequences, ATG codons and 5’ homology regions. All PCR amplified fragments used in the cloning procedures were sequenced to confirm that no unwanted mutations are present. SLiCE extract was prepared as described in Zhang et al.8 The PPY strain used for SLiCE preparation was kindly provided by Dr. Yongwei Zhang (Department of Cell Biology, Albert Einstein College of Medicine, New York City).

1. Cloning of the four major SHANK2 isoforms into pENTR2B vector for N-terminal tags
   a) SHANK2-SH3 – construct was generated before in our lab.
   b) SHANK2E – N-terminal Ank repeats region was amplified with primers Shank2E_F and Shank2E_R using cDNA from whole human brain as matrix. Generated PCR product was seamlessly joined to pENTR2B-SHANK2-SH3 (previously linearized using SalI restriction enzyme) based on short end homology via SLiCE reaction as described in Zhang et al.8

| Primer       | Sequence (5'-3')                                 |
|--------------|--------------------------------------------------|
| Shank2E_F    | TACAAAAAGCAGGCTGGCCGCAGCCCCACATGCCAG             |
| Shank2E_R    | CACGTAGCTCCCCAATGGTC                             |

2. Cloning of the SHANK2E isoform into pENTR2B vector for C-terminal tags
   a) SHANK2-SH3 – the coding sequence of SHANK2-SH3 from the N-terminal construct was excised with SalI and KpnI restriction enzymes. pENTR2B vector was PCR amplified with primers SHANK2PDZC_1 and SHANK2PDZC_2. First primer provided Kozak sequence, in frame ATG codon and 18 bases homology to the coding sequence. Second primer provided 18 bases homology to the region immediately before the stop codon in the coding sequence. pENTR2B used as matrix was destroyed via DpnI digestion after the amplification step. Both generated PCR product and restriction fragment were joined together via SLiCE reaction generating construct with Kozak sequence, ATG codon and coding sequence that is in frame with the 3’ att site as recommended by the Gateway cloning manual.
   b) SHANK2E – this isoform was generated as the N-terminal construct using SHANK2-SH3 C-terminal construct and primers Shank2EC_F and Shank2EC_R

| Primer       | Sequence (5'-3')                                 |
|--------------|--------------------------------------------------|
| Shank2SH3C_F | GTAGCTCCAATGGTCTGACTGACCATGGTGCCCCAGCTTGTTTTGTAC |
| Shank2SH3C_R | AAACAGCTGCTGCAGACTAGACGCCCCACTTCTTGTAC           |
| Shank2EC_F   | TACAAAAAGCAGGCTGGCAGCCCCACATGCGCAGCCCCACATCCAG  |
| Shank2EC_R   | CACGTAGCTCCCCAATGGTC                             |

3. Site-directed mutagenesis

All four tested variants were introduced into pENTR2B-SHANK2-SH3 construct for N-terminal tags using the QuikChange Lightning Site-directed Mutagenesis Kit (Stratagene) according to the manual. Used primers are listed in the table below. Nucleotides that differ from the wild type sequence are colored in red. Mutagenesis efficiency for variant R958S was significantly lower due to a sable loop structure in the primers (underlined bases). This required screening of more colonies to obtain positive ones. Mutated and confirmed constructs were used in a second step to generate pENTR2B-SHANK2E versions of the variants via the mechanism described in 1.b) and c).
4. Sequencing primers for the SHANK2E isoform generated in this study
Standard sequencing primers ENTR_F and ENTR_R were used for pENTR2B vector. Coding sequence of SHANK2-SH3 was sequenced as described in Berkel et al. Primers used for sequencing of the additional sequences in SHANK2E are listed in the table below.

| Primer | Sequence (5'-3') |
|--------|-----------------|
| S610Y_A | CTACACCGTGGGCTACTATGACAGCTTCG |
| S610Y_B | CGAAGCTGTCATAGTAGCCACCGTGTAAG |
| R958S_A | CAGGAGAGCTGGGACAGCTACTCTCCGTGAC |
| R958S_B | GTCCAAGGAGTAGTGCAGTCCGCTCTCCG |
| P1119T_A | GGCACCAGGCACCCGAGAGCGCCGGCC |
| P1119T_B | GGCCGCGCTCTGGTGCCGCTGGCC |
| A1731S_A | GTCTGGACCACTCTCTCTCTCCGC |
| A1731S_B | GCCGGAGAAGGAGAGGGTGACAGCACAC |

5. Brief description of all DEST vectors used in the present study
a) pN-mCherry-DEST – this vector was created before in our lab. It provides N-terminal mCherry tag which is in frame with the 5' att site. Expression is driven by CMV promoter.

b) pN-YFP-DEST – this vector was a kind gift from Stefan Wiemann. It provides N-terminal YFP tag which is in frame with the 5' att site. Expression is driven by CMV promoter.

c) pC-YFP-DEST – this vector was kind gift from Stefan Wiemann. It provides C-terminal YFP tag which is in frame with the 3' att site. Expression is driven by CMV promoter. ENTR clones should provide Kozak sequence and ATG codon and also they have to lack STOP codon at the end of the coding sequence.

d) pCS2-DEST – this vector was generated for the purposes of the present study. DEST cassette was PCR amplified from DEST 12.2 vector and ligated into pCS2 digested with XhoI. Generated vector provided tag free expression driven by CMV promoter.
Supplementary References

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