Psychiatry Research 275 (2019) 94–99

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Genetic variation of *UBE3A* is associated with schizotypy in a population of typical individuals

Iiro Salminen[a,*], Silven Read[a], Pete Hurd[b], Bernard Crespi[a]

[a] Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada
[b] Department of Psychology and Centre for Neuroscience, University of Alberta, Edmonton, Canada

ARTICLE INFO

Keywords:
Angelman syndrome
Prader-Willi syndrome
Autism spectrum disorder
Schizophrenia
15q11-q13 duplication
Genomic imprinting

ABSTRACT

The maternally expressed imprinted gene UBE3A has been implicated in autism, schizophrenia and psychosis. The phenotype of Angelman syndrome, caused by loss of UBE3A expression, involves autism spectrum traits, while Prader-Willi syndrome, where the genotype of maternal disomy increases dosage of UBE3A, shows high penetrance for the development of psychosis. Maternal duplications of the 15q11-q13 chromosome region that overlap the imprinted region also show an association with schizophrenia, further implying a connection between increased dosage of UBE3A and the development of schizophrenia and psychosis. We phenotyped a large population of typical individuals for autism spectrum and schizotypal traits and genotyped them for a set of SNPs in UBE3A. Genetic variation of rs732739, an intronic SNP tagging a large haplotype spanning nearly the entire range of UBE3A, was significantly associated with variation in total schizotypy. Our results provide an independent line of evidence, connecting the imprinted UBE3A gene to the schizophrenia spectrum.

1. Introduction

The imprinted gene *UBE3A*, encoding an E3 ubiquitin ligase protein, is expressed only from the maternal copy in neurons (Albrecht et al., 1997). The silencing of the paternally inherited copy is mediated by a long RNA transcript, which also contains a sequence complementary to *UBE3A* (*UBE3A* antisense transcript, UBE3A-ATS) and initiates from the unmethylated, paternally inherited copy of the 15q11-q13 imprinting center near the SNURF-SNRPN gene. In the maternally inherited copy, the corresponding imprinting center is methylated, preventing the expression of the UBE3A-ATS and the subsequent paternally expressed genes imprinted genes (reviewed in Mabb et al., 2011).

The E3 ubiquitin ligases co-operate with specific E2 ubiquitin ligase proteins to identify a range of target proteins, which are subsequently marked for degradation, activation or relocation in the cell (LaSalle et al., 2015). The gene is expressed in multiple alternatively spliced mRNAs and three different protein isoforms (Yamamoto et al., 1997), which have functions in neuron differentiation and development, as suggested by mouse models (reviewed in Lopez et al., 2019). Thus, *UBE3A* affects neuron development and function as well as behavioral and psychological phenotypes via multiple different mechanisms, involving both direct and indirect interactions with specific target proteins (Lopez et al., 2019).

UBE3A is located in a ~ 2Mb imprinted domain in the 15q11-q13 chromosome region, which is punctuated by several chromosomal break points (Nicholls and Knepper, 2001). The 15q11-q13 chromosome region is particularly well-known for a 'sister pair' of neurodevelopmental syndromes, which involve lack of expression for paternally expressed genes in Prader-Willi Syndrome (PWS) and a lack of expression for the maternally expressed *UBE3A* gene in Angelman Syndrome (AS), respectively (Cassidy et al., 2012; Margolis et al., 2015). The two syndromes show distinct behavioral phenotypes. AS subjects typically show a sociable and happy disposition with frequent laughter (Margolis et al., 2015), while autism spectrum disorders (ASD) and behaviors typical of the autism spectrum are also highly prevalent (Bonati et al., 2007). In comparison, PWS involves rapid mood fluctuations and considerable negative affect (Sinnema et al., 2011a), with psychiatric disorders including depression, bipolar disorder and most prominently, affective psychoses being highly prevalent, especially for the genotype of maternal disomy (Yang et al., 2013). Given that AS involves loss of expression of *UBE3A* (Albrecht et al., 1997; Kishino et al., 1997), while PWS due to uniparental disomy involves overexpression of this gene (Bittel et al., 2003; Hogart et al., 2008), *UBE3A* may be linked to such alterations of social behavior in the context of autism spectrum and psychotic-affective conditions.

Lack of expression for *UBE3A* has been implicated in risk for ASDs from two lines of evidence. Firstly, AS involves a high prevalence of ASDs, with estimates ranging from ~40 to 80% of affected individuals

* Corresponding author.
E-mail address: isalmine@sfu.ca (I. Salminen).

https://doi.org/10.1016/j.psychres.2019.03.019
Received 5 February 2019; Received in revised form 4 March 2019; Accepted 12 March 2019
Available online 14 March 2019
0165-1781/

(Bonati et al., 2007; Peters et al., 2004; Trillingsgaard and Østergaard, 2004). Behaviors relevant to the autism spectrum in AS include deficits in social interactions, repetitive and stereotypical behaviors, as well as deficits in communication, partly attributable to developmental delay with near or complete lack of speech. A recent study showed that AS individuals diagnosed with autism spectrum traits in early childhood showed later improvements in both expressive and receptive language skills, while the levels of autism spectrum traits did not change (Mertz et al., 2014). Thus, the behavioral phenotype of AS reflects the broad characteristics typical of ASDs involving developmental delay, which indicates a connection between lack of expression for *UBE3A* and the development of autism spectrum traits.

Secondly, several studies have investigated associations of markers within *UBE3A* with idiopathic autism, with mixed results. Cook et al. (1998) found that several microsatellite markers in the genetic region of *UBE3A*, including *D15S122* (chr15:25434850–25435145, repeating sequence AC), located at the 5′ untranslated region of *UBE3A,* did not show any pattern of preferential transmission in autism families. Nurmi et al. (2001) studied a population of families where at least two children had a diagnosis of autism and found that the marker *D15S122* showed a pattern of preferential transmission among autism families. The microsatellite marker was analyzed as 12 different alleles with lengths ranging from 137-bp to 163-bp, with the 145-bp, 147-b and 149-bp alleles covering the majority of the variation. A 155-bp allele showed a significant maternal effect in transmission to affected siblings, while a 147-bp allele showed a significant negative association with autism. A later study by the same group, using six intronic SNP markers within *UBE3A* did not find any significant pattern of preferential transmission in autism families (Nurmi et al., 2003). Guffanti et al. (2011) found that an allele of the microsatellite marker *D15S122* showed preferential under transmission to affected children, partially replicating the earlier results of Nurmi et al. (2001). Here, the microsatellite marker was analyzed as four classes of different alleles designated as 222-bp, 224-bp, 226-bp and other lengths, with the three main alleles covering the majority of the variation. Two other studies using SNP markers within *UBE3A* did not find any significant associations between *UBE3A* and ASDs (Kato et al., 2008; Kim et al., 2008). While these studies suffer from limited sample sizes and a lack of statistical power, their results suggest that genetic variation affecting *UBE3A*, most notably rare haplotypes tagged by the marker *D15S122,* may be associated with an increased risk for ASDs.

In contrast to the link between lack of expression for *UBE3A* and AS, increased dosage of *UBE3A* has been associated with psychotic-affective spectrum conditions from several independent lines of evidence. Firstly, PWS individuals with the genotype of maternal disomy show approximately 2- to 3-fold higher expression of *UBE3A,* as shown in studies with cDNA microarrays from cell lines of individuals with different genotypes of PWS (Bittel et al., 2003; Hogart et al., 2008); as noted above, PWS subjects with maternal disomy also show a significantly increased risk for psychotic and bipolar disorders as compared to individuals with deletions (Yang et al., 2013). Both case studies and several independent studies with large cohorts (references in Crespi et al. (2018) supplementary table) have shown that individuals with the genotype of maternal disomy show a high prevalence of affective psychoses, involving mainly paranoid ideation and auditory hallucinations (Sinnema et al., 2011b; Soni et al., 2007; Verhoeven et al., 2003; Vogels et al., 2004). Associations between the genotype of maternal disomy, with two maternally inherited copies of *UBE3A* and development of psychosis in PWS, suggest that increased dosage of *UBE3A* may contribute to the development of psychotic-affective phenotypes and conditions.

Secondly, similarly to the genotype of maternal disomy in PWS, maternal duplications of the 15q11-q13 chromosome region involve a ~1.5- to 3-fold increase in expression of *UBE3A* as shown by studies with mRNA derived from post-mortem brain tissues and cell lines (Herzing et al., 2002; Scoles et al., 2011). Studies of large cohorts of individuals with schizophrenia have indicated that only maternal duplications (and not paternal duplications) of the 15q11-q13 region increase the risk of schizophrenia. Ingason et al. (2011) genotyped cohorts of schizophrenia patients from several European countries and found significant overrepresentation of maternal duplications among the schizophrenia group as compared to the controls. Similarly, a more comprehensive follow-up study including large cohorts from both ASDs and developmental delay, as well as schizophrenia, found that only maternal duplications mediated an increased risk for schizophrenia, while both paternal and maternal duplications were found to increase the risk for ASDs (Isles et al., 2016). The duplications of the schizophrenia patients did not show consistent patterns with regard to the size of the duplication, but the imprinted region between break point 2 and break point 3, which contains *UBE3A*, was common to all duplications associated with schizophrenia. As only maternal duplications of the region showed a consistent association with schizophrenia, the authors of both studies (Ingason et al., 2011; Isles et al., 2016) suggested that maternally expressed genes, and in particular *UBE3A,* are likely to be involved in mediating the risk for schizophrenia.

Thirdly, Noor et al. (2015) found that a microduplication encompassing only *UBE3A* had segregated for several generations in a family line, and that maternal inheritance of the duplication was associated with developmental delay, anxiety, depression, and schizophrenia in family members. Furthermore, cell line experiments with a patient's fibroblasts showed approximately twofold expression levels of *UBE3A* as compared to controls, with no effect on the expression of surrounding genes, more directly implicating increased expression of *UBE3A* in connection with depression and schizophrenia.

Duplications of the entire 15q11-q13 chromosome region have also been shown to be associated with ASDs, which has been suggested to indicate a link between increased dosage of *UBE3A* and autism (Hogart et al., 2010; Vatsa and Jana, 2018). Isles et al. (2016) analyzed large cohorts of individuals with ASD and developmental delay from several previously published studies along with new data and found that both paternal and maternal duplications overlapping with the region between breakpoints 2 and 3, or alternatively with breakpoints 1 and 3 in the 15q11-q13 chromosome region, showed significant associations with a phenotype involving ASD with developmental delay and multiple congenital anomalies, with ~ 50% penetrance for maternal and ~20% penetrance for paternal duplications. Given that these duplications overlap with both imprinted and non-imprinted domains, several different genes in the chromosome region may be involved in mediating the risk for ASDs.

Several mouse model studies of *UBE3A* show notable effects of varying expression of this gene in neuron development and function (Dindot et al., 2007; Fink et al., 2017; Greer et al., 2010; Jiang et al., 1998; Kaphzan et al., 2011; Miao et al., 2013; Sun et al., 2015), whereby deletions involve opposite social phenotypes as compared to increased dosage. In particular, model mice with a lack of expression for *UBE3A* showed significantly increased amounts of ultrasonic vocalization as compared to controls (Jiang et al., 2010; Stoppel and Anderson, 2017) and prolonged social interest in an interaction test with a novel mouse of same sex, as compared to controls (Stoppel and Anderson, 2017). In comparison, two studies found that transgenic mice with two additional copies of *UBE3A* showed a reduced amount of ultrasonic vocalizations during interactions with a same-sex pair as compared to controls (Krishnan et al., 2017; Smith et al., 2011), and did not show a typical preference for interaction with a caged mouse of same sex (Krishnan et al., 2017). These results suggest that opposite imbalances in dosage of *UBE3A* may be linked to opposite alterations in aspects of social behavior.

A consistent pattern across studies thus indicates that increased dosage of *UBE3A*, as shown with both maternal disomy in PWS and maternal 15q11-q13 duplications, may be mediating the risk for schizophrenia, and related psychotic-affective conditions, in clinical

populations. In contrast, lack of expression for *UBE3A* may be linked with ASDs, as implicated by the high prevalence of ASDs in AS, which involves a lack of expression for *UBE3A* in neurons. Furthermore, haplotypes of *UBE3A* may mediate an increased risk of ASDs.

As both psychotic-affective spectrum conditions and ASDs can be considered as genetic and neuropsychiatric continuums grading into typical functioning and social behavior (David, 2010; Robinson et al., 2016; van Os et al., 2009), it can be further postulated that genetic variation in *UBE3A* may affect phenotypes of autism spectrum traits or schizotypy in non-clinical populations. We have characterized variation in both autism spectrum traits as well as schizophrenia spectrum traits in a large population of typically developing individuals and genotyped them for a set of SNPs in the *UBE3A* genetic region. We hypothesized that genetic variation in *UBE3A* may thus be associated with variation in schizotypal traits, autism spectrum traits or both, due to effects on mRNA levels, post-transcriptional regulation, or activity of the protein, and other processes.

2. Methods

The study was approved by the ethics boards at both the University of Alberta (Pro00015728) and Simon Fraser University (2010s0554), with all participants providing prior written informed consent. We collected questionnaire and DNA data from 507 undergraduate students (285 females and 222 males) of Caucasian ancestry. Forms and levels of schizotypal traits were quantified with the Schizotypal Personality Questionnaire - Brief Revised (SPQ-BR) (Cohen et al., 2010). The questionnaire consists of 32 items using a 5-point Likert scale ranging from 'strongly disagree' to 'strongly agree'. The questions are further divided across seven subscales of personality traits and social behavior which include 1) ideas of reference, 2) constricted affect, 3) eccentric behavior, 4) social anxiety, 5) magical thinking, 6) odd speech and 7) unusual perceptions, and sum together to total schizotypy. The Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) was used to quantify the extent that individuals endorsed personality traits and behavior associated with the autistic spectrum. The questionnaire is comprised of 50 items and assesses personality traits and social behavior across five domains including 1) communication, 2) social skills, 3) imagination, 4) attention to detail and 5) attention switching, with a total AQ score as the sum.

A set of six SNPs was initially chosen to characterize the genetic variation of *UBE3A*. In particular, rs732739 (chr15:25434520, C/T, in hg38, 2013) is located within the first intron of *UBE3A*, 330-bp downstream from the starting site of *D15122*, a microsatellite marker, which has been previously shown to display patterns of preferential transmission in autism families (Guffanti et al., 2011; Nurmi et al., 2001). The marker is also in partial linkage disequilibrium (D′ = 1.0, r^2 = 0.54, in CEU 1000genomes) with rs189782611 (chr15:25434997, C/G) located within *D15S122*. (We used both D' and r^2 to measure LD. D′ indicates how often an allele of a given marker is inherited together with the associated allele of the other marker, while r^2 is also affected by the allele frequencies. Therefore, a pair of genetic markers showing high D′ with low r^2 do not correspond to the same underlying genetic variation). Two SNPs, rs11630723 (chr15:25442741, A/T) and rs11161178 (chr15:25444144 A/G), were selected to target the promoter region of *UBE3A*, while rs1041933 (chr15:25409602, C/T) rs17115577 (chr15:25422613, A/C) and rs7176461 (chr15:25419872, A/C) were selected to target genetic variation in the untranslated region 5′ of *UBE3A*. As rs11630723 and rs7176461 were reported to be in complete LD with other genetic markers in our study, based on data from individuals of Caucasian descent (rs11161178 and 11630723, r^2 = 1.0, D′ = 1.0; rs732739 and rs7176461 r^2 = 1.0, D′ = 1.0), rs11630723 and rs7176461 were not included in the full analysis; in each case, we analyze data and report results from the markers with larger sample sizes. Unadjusted p-values for all six SNPs are provided in supplementary files. As genetic variation may differ between populations, our analyses were limited to students of Caucasian descent, based on demographic data collected with the questionnaires. The SNPs in our study population showed nearly identical allele frequencies as compared to previously reported results with populations of Caucasian descent.

Fluorophore-labelled primers for SNPs rs732739, rs1041933, rs17115577, rs1716461, rs11630723 and rs11161178 were used in TaqMan genotyping using a Roche Light-Cycler 96 Real-Time PCR machine. Fluorescence data were analysed under Endpoint Genotyping with the LightCycler 96 Software v. 1.1.0.1320 (2011) and genotyping success varied between 96.8 to 99.0%. Each of the markers was in Hardy-Weinberg equilibrium, as measured by the Fisher Exact test (rs1041933, $p = 0.07$; rs17115577, $p = 1$; rs732739, $p = 0.21$; rs7176461, $p = 0.34$; rs11630723, $p = 0.39$; rs11161178, $p = 0.45$).

Under the simplest model of imprinting, only the maternally inherited allele would be expected to affect the expression of a maternally expressed imprinted gene. Thus, at an imprinted locus with the alleles A and a, Aa and aA heterozygotes, differing by parental origin, would be expected to show distinct phenotypes. However, as our data does not include genotypes of family trios, and the silencing of paternal copy of *UBE3A* is not mediated by methylation of the promoter area (Mabb et al., 2011), we are unable to determine the distinct genotypes of the heterozygous individuals. We have conducted an additional analysis relevant to this issue, by comparing the phenotypic variation of the two homozygous groups, showing the effects of both alleles unambiguously. T-tests and ANOVAs for phenotypic AQ and SPQ differences were calculated for each SNP under four genetic models: [1] Codominant, (common homozygotes versus heterozygotes versus rare homozygotes), [2] Dominant, (common homozygotes versus heterozygotes + rare homozygotes), [3] Recessive (common homozygotes + heterozygotes versus rare homozygotes) and [4] Homozygotes-only (common homozygotes versus rare homozygotes) in R v. 3.5.1. (2018). As the genetic models are not independent of each other, corrections for multiple testing were based on tests on four genetic markers, adjusted separately for each of our initial hypotheses on schizotypy and autism spectrum traits. Further adjustments accounting for tests on both AQ and SPQ scales and tests on different genetic models were also performed (data not shown). False Discovery Rate (FDR) adjustments using the 0.05 level of significance were performed with the p.adjust feature in the R stats package (v. 3.5.1)

3. Results

The total schizotypy score showed significant differences between individuals with different genotypes of rs732739 under the codominant (rs732739 unadjusted $p = 0.018$), and the recessive model (rs732739 unadjusted $p = 0.0055$) (Table 1). In addition, the total schizotypy score showed significant differences in our additional analysis, comparing the two homozygote genotypes (unadjusted $p = 0.0057$, see supplementary Table 2). After FDR adjustment for tests on four genetic markers, only the results with the recessive and homozygotes-only models for rs732739 remained significant. These results also remained significant after twofold adjustments for tests using both total AQ and total SPQ-BR scores. Comparison of the mean SPQ-BR scores indicates that the rare homozygote (TT) individuals on average had about 18% lower SPQ-BR scores than individuals with other genotypes. The total AQ scores did not show significant results on any of the markers, but the subscale of social skills showed nominally significant differences (unadjusted $p = 0.03$) with the dominant model on rs11161178 (supplementary Table 1). On SPQ-BR subscales, rs732739 showed nominally significant differences on the subscales of ideas of reference, constricted affect, eccentric behavior, interpersonal features, and disorganized features, on codominant, imprinted and recessive models (supplementary Table 1), but none of the associations between genetic markers and AQ or SPQ-BR subscales remained significant after FDR adjustments.

Table 1
Mean ± s.d. for Autism Quotient (AQ) and Schizotypal Personality Questionnaire - Brief Revised (SPQ-BR) scores for each genotype of the four UBE3A SNP markers included in the full analysis. Unadjusted p-values (p) for ANOVA tests on three genetic models (Codominant, Dominant and Recessive) are shown first, while *p*-values corrected by a false discovery rate of 0.05 (p_{fdr}) are also provided. The FDR correction was based on tests on four different markers, adjusted separately for each genetic model and phenotypic variable (degrees of freedom for each test: 4, see methods for reasoning).

SNP	Genotype1(*n*)	Genotype2(*n*)	Genotype3(*n*)	Cod (p/p_{fdr})	Dom (p/p_{fdr})	Rec (p/p_{fdr})
rs1041933	TT (284)	CT (187)	CC (27)			
AQ	16.93 ± 5.58	16.80 ± 5.22	14.96 ± 5.58	0.201 / 0.402	0.467 / 0.801	0.076 / 0.17
SPQ-BR	86.63 ± 14.80	86.50 ± 15.94	82.63 ± 14.80	0.495 / 0.660	0.922 / 0.922	0.245 / 0.327
rs17115577	CC (446)	AC (45)	AA (4)			
AQ	16.87 ± 5.50	16.18 ± 4.61	16.25 ± 8.06	0.707 / 0.707	0.405 / 0.801	0.840 / 0.840
SPQ-BR	85.86 ± 16.25	87.69 ± 12.30	90.25 ± 8.06	0.664 / 0.664	0.395 / 0.922	0.597 / 0.597
rs732739	CC (381)	CT (111)	TT (8)			
AQ	16.77 ± 5.41	17.08 ± 5.56	13.50 ± 4.90	0.198 / 0.402	0.898 / 0.898	0.085 / 0.17
SPQ-BR	86.10 ± 15.58	87.00 ± 16.47	70.63 ± 15.09	0.018 / 0.072	0.903 / 0.922	0.006 / 0.02
rs11161178	GG (302)	172 (AG)	28 (AA)			
AQ	16.65 ± 5.44	17.13 ± 5.57	15.54 ± 5.15	0.313 / 0.417	0.601 / 0.801	0.226 / 0.301
SPQ-BR	85.61 ± 15.87	87.27 ± 16.17	82.11 ± 13.67	0.226 / 0.452	0.515 / 0.922	0.184 / 0.327

4. Discussion

Our primary results are twofold. First, rs732739, an intronic marker within *UBE3A,* shows a significant FDR-adjusted association with total schizotypy, and nominally significant associations with several subscales, under codominant and recessive models. These results indicate that genetic variation of *UBE3A* segregating among non-clinical populations affects levels of schizotypy within the range of typical variation.

These findings fit with previously discussed evidence showing that *UBE3A* appears to mediate the risk for development of psychosis in PWS and the risk for schizophrenia associated with maternal 15q11-q13 duplications. Our results show nominally significant associations with the subscales of interpersonal and disorganized schizotypy, with the former reflecting constricted affect and paranoid ideation in social contexts, and the latter reflecting endorsement of statements regarding odd speech and eccentric behaviors. The phenotypes of psychosis in PWS similarly involve, most commonly, paranoid ideation in the forms of second-person hallucinations and persecutory delusions (Soni et al., 2008), as well as mood swings, confusion and obsessive rituals (Soni et al., 2008; Verhoeven et al., 2003), which resemble disorganized aspects of schizophrenia spectrum disorders. Thus, our results with the maternally expressed *UBE3A,* which shows about twofold increase in expression levels with the genotype of maternal disomy in PWS (Bittel et al., 2003; Hogart et al., 2008), appear consistent with previous clinical findings as regards to psychiatric traits mediated by *UBE3A*.

Second, we note that rs732739, the marker associated with total schizotypy in our study, is linked (rs189782611, D′ = 1.0, r^2 = 0.54, located within 330-bp) with a genetic marker located within *D15S122*, a microsatellite marker associated with autism in two transmission disequilibrium studies (Guffanti et al., 2011; Nurmi et al., 2001). However, as neither our study nor any previously published study included genotyping of the *D15S122* microsatellite along with rs732739, it is not possible with available data to determine if a particular allele of rs732739 is associated with a *D15S122* microsatellite allele of a certain size. Future work could usefully address this issue.

A large region of the *UBE3A* gene interacts with the *UBE3A* antisense transcript, which as previously mentioned, regulates the silencing of the paternal copy of *UBE3A* in neurons (Mabb et al., 2011). As shown by a mouse model study in which a mutation in the maternally inherited copy of *Ube3a* increased the expression of the Ube3a-ATS as compared to controls (Landers et al., 2005), the paternally and maternally inherited alleles may interact with each other directly. More recently, a cell model study found that a border element consisting of the *IPW* and *PWAR1* genes was necessary for the imprinting of the paternal allele in nerve cells, as removal of this region led to increased expression of UBE3A-ATS in AS-derived nerve cells, without repressing the expression of *UBE3A* from the paternally inherited allele (Hsiao et al., 2019). Thus, genetic variation of regions overlapping the antisense transcript may affect the expression of *UBE3A* via the paternally inherited allele in some manner. Such a mechanism may have implications for tests of the hypothesis that increased expression of *UBE3A* is expected to mediate the expression of schizotypy, while reduced expression of *UBE3A* influences the expression of autism spectrum traits.

The genetic marker showing our main result, rs732739, is strongly linked with genetic markers within the *UBE3A* promoter region (D′ = 1.0 r^2 = 0.947 with rs72697799, located approximately 1000-bp upstream of exon 1, at chr15:25440750) As previously discussed, *UBE3A* is alternatively spliced in several tissue types, including neurons (LaSalle et al., 2015; Yamamoto et al., 1997) and the longer forms of *UBE3A* mRNA include 5′-UTR sequences comprising of the first exons, which are not translated to the amino acid sequence in any of the three different isoforms of *UBE3A*. While the specific purpose of mechanisms regulating alternative splicing and translation of *UBE3A* is not known in humans (LaSalle et al., 2015; Lopez et al., al.,2019), 5′-UTR sequences may include ribosomal binding sites and mRNA secondary structures, which further regulate translation events (Araujo et al., 2012). Thus, both *D15S122* and rs732739 may tag genetic variation linked to promoter and 5′-UTR regions of *UBE3A* that regulates expression. The SNP rs732739 indeed represents a highly-significant expression QTL for muscle tissues (Lonsdale et al., 2013), indicating that the marker is functionally associated with expression-level variation in *UBE3A*. Further studies are needed on mechanisms whereby rs732739 and linked regions may mediate brain development and function via regulation of gene expression, alternative splicing, protein interactions or other mechanisms, with regard to both autism and psychotic-affective spectrum disorders.

Funding

The project was funded by NSERC 2014-06505.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.03.019.

References

Albrecht, U., Sutcliffe, J.S., Cattanach, B.M., Beechey, C.V., Armstrong, D., Eichele, G., Beaudet, A.L., 1997. Imprinted expression of the murine Angelman syndrome gene, *UBE3A*, in hippocampal and Purkinje neurons. Nat. Genet. 17, 75–78. https://doi.org/10.1038/ng0997-75.

Araujo, P.R., Yoon, K., Ko, D., Smith, A.D., Qiao, M., Suresh, U., Burns, S.C., Penalva, L.O.F., 2012. Before it gets started: regulating translation at the 5′ UTR. Comp. Funct. Genom. 2012, 1–8. https://doi.org/10.1155/2012/475731.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., Clubley, E., 2001. The Autism-

Spectrum Quotient (AQ): evidence from. J. Autism Dev. Disord. 31, 5–17. https://doi.org/10.1023/A:1005653411471.
Bittel, D.C., Kibiryeva, N., Talebizadeh, Z., Butler, M.G., 2003. Microarray analysis of gene/transcript expression in Prader-Willi syndrome: deletion versus UPD. J. Med. Genet. 40, 568–574. https://doi.org/10.1136/JMG.40.8.568.
Bonati, M.T., Russo, S., Finelli, P., Valsecchi, M.R., Cogliati, F., Cavalleri, F., Roberts, W., Elia, M., Larizza, L., 2007. Evaluation of autism traits in Angelman syndrome: a resource to unfold autism genes. Neurogenetics 8, 169–178. https://doi.org/10.1007/s10048-007-0086-0.
Cassidy, S.B., Schwartz, S., Miller, J.L., Driscoll, D.J., 2012. Prader-Willi syndrome. Genet. Med. 14, 10–26. https://doi.org/10.1038/gim.0b013e31822bead0.
Cohen, A.S., Matthews, R.A., Najolia, G.M., Brown, L.A., 2010. Toward a more psychometrically sound brief measure of schizotypal traits: introducing the SPQ-brief revised. J. Pers. Disord. 24, 516–537. https://doi.org/10.1521/pedi.2010.24.4.516.
Cook, E.H., Courchesne, R.Y., Cox, N.J., Lord, C., Gonen, D., Guter, S.J., Lincoln, A., Nix, K., Haas, R., Leventhal, B.L., Courchesne, E., 1998. Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. Am. J. Hum. Genet. 62, 1077–1083.
Crespi, B., Read, S., Salminen, I., Hurd, P., 2018. A genetic locus for paranoia. Biol. Lett. 14, 20170694. https://doi.org/10.1098/rsbl.2017.0694.
David, A.S., 2010. Why we need more debate on whether psychotic symptoms lie on a continuum with normality. Psychol. Med. 40, 1935–1942. https://doi.org/10.1017/S0033291710000188.
Dindot, S.V., Antalffy, B.A., Bhattacharjee, M.B., Beaudet, A.L., 2007. The Angelman syndrome ubiquitin ligase localizes to the synapse and nucleus, and maternal deficiency results in abnormal dendritic spine morphology. Hum. Mol. Genet. 17, 111–118. https://doi.org/10.1093/hmg/ddm288.
Fink, J.J., Robinson, T.M., Germain, N.D., Sirois, C.L., Bolduc, K.A., Ward, A.J., Rigo, F., Chamberlain, S.J., Levine, E.S., 2017. Disrupted neuronal maturation in Angelman syndrome-derived induced pluripotent stem cells. Nat. Commun. 8, 1–14. https://doi.org/10.1038/ncomms15038.
Greer, P.L., Hanayama, R., Bloodgood, B.L., Mardinly, A.R., Lipton, D.M., Flavell, S.W., Kim, T.-K., Griffith, E.C., Waldon, Z., Maehr, R., Ploegh, H.L., Chowdhury, S., Worley, P.F., Steen, J., Greenberg, M.E., 2010. The Angelman syndrome protein *UBE3A* regulates synapse development by ubiquitinating arc. Cell 140, 704–716. https://doi.org/10.1016/j.cell.2010.01.026.
Guffanti, G., Lievers, L.S., Bonati, M.T., Marchi, M., Geronazzo, L., Nardocci, N., Estienne, M., Larizza, L., Macciardi, F., Russo, S., 2011. Role of *UBE3A* and ATP10A genes in autism susceptibility region 15q11-q13 in an Italian population: a positive replication for *UBE3A*. Psychiatry Res. 185, 33–38. https://doi.org/10.1016/j.psychres.2010.04.057.
Herzing, L.B.K., Cook, J.E.H., Ledbetter, D.H., 2002. Allele-specific expression analysis by RNA-FISH demonstrates preferential maternal expression of *UBE3A* and imprint maintenance within 15q11- q13 duplications. Hum. Mol. Genet. 11, 1707–1718. https://doi.org/10.1093/hmg/11.15.1707.
Hogart, A., Leung, K.N., Wang, N.J., Wu, D.J., Driscoll, J., Vallero, R.O., Schanen, N.C., LaSalle, J.M., 2008. Chromosome 15q11-13 duplication syndrome brain reveals epigenetic alterations in gene expression not predicted from copy number. J. Med. Genet. 46, 86–93. https://doi.org/10.1136/jmg.2008.061580.
Hogart, A., Wu, D., LaSalle, J.M., Schanen, N.C., 2010. The comorbidity of autism with the genomic disorders of chromosome 15q11.2-q13. Neurobiol. Dis. 38, 181–191. https://doi.org/10.1016/j.nbd.2008.08.011.
Hsiao, J.S., Germain, N.D., Wilderman, A., Stoddard, C., Wojenski, L.A., Villafano, G.J., Leighton, C., Cotney, J., Chamberlain, S.J., 2019. A bipartite boundary element restricts *UBE3A* imprinting to mature neurons. Proc. Natl. Acad. Sci. 116 (6), 2181–2186. https://doi.org/10.1073/pnas.1815279116.
Ingason, A., Kirov, G., Psych, M.R.C., Giegling, I., Hansen, T., Isles, A.R., Jakobsen, K.D., Kristinsson, K.T., Le Roux, L., Gustafsson, O., Craddock, N., Psych, F.R.C., Möller, H.-J., Mcquillin, A., Muglia, P., Cichon, S., Rietschel, M., Ophoff, R.A., Djurovic, S., Andreassen, O.A., Pietiläinen, O.P.H., Peltonen, L., Dempster, E., Collier, D.A., Clair, D.S., Rasmussen, H.B., Glenthøj, Y., Kiemeney, L.A., Franke, B., Tosato, S., Bonetto, C., Saemundsen, E., Hreidarsson, S.J., Nöthen, M.M., Gurling, H., O'donovan, M.C., Owen, M.J., Sigurdsson, E., Petursson, H., Stefansson, H., Rujescu, D., Stefansson, K., Werge, T., 2011. Maternally derived microduplications at 15q11-q13: implication of imprinted genes in psychotic illness Europe PMC funders group. Am. J. Psychiatry 168, 408–417. https://doi.org/10.1176/appi.ajp.2010.09111660.
Isles, A.R., Ingason, A., Lowther, C., Walters, J., Gawlick, M., Stöber, G., Rees, E., Martin, J., Little, R.B., Potter, H., Georgieva, L., Pizzo, L., Ozaki, N., Aleksic, B., Kushima, I., Ikeda, M., Iwata, N., Levinson, D.F., Gejman, P.V., Shi, J., Sanders, A.R., Duan, J., Willis, J., Sisodiya, S., Costain, G., Werge, T.M., Degenhardt, F., Giegling, I., Rujescu, D., Hreidarsson, S.J., Saemundsen, E., Ahn, J.W., Ogilvie, C., Girirajan, S.D., Stefansson, H., Stefansson, K., O'Donovan, M.C., Owen, M.J., Bassett, A., Kirov, G., 2016. Parental origin of interstitial duplications at 15q11.2-q13.3 in schizophrenia and neurodevelopmental disorders. PLoS Genet. 12, 1–15. https://doi.org/10.1371/journal.pgen.1005993.
Jiang, Y., Armstrong, D., Albrecht, U., Atkins, C.M., Noebels, J.L., Eichele, G., Sweatt, J.D., Beaudet, A.L., 1998. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. Neuron 21, 799–811. https://doi.org/10.1016/S0896-6273(00)80596-6.
Jiang, Y., Pan, Y., Zhu, L., Landa, L., Yoo, J., Spencer, C., Lorenzo, I., Brilliant, M., Noebels, J., Beaudet, A.L., 2010. Altered ultrasonic vocalization and impaired learning and memory in Angelman syndrome mouse model with a large maternal deletion from *UBE3A* to Gabrb3. PLoS One 5, e12278. https://doi.org/10.1371/journal.pone.0012278.
Kaphzan, H., Buffington, S.A., Jung, J.I., Rasband, M.N., Klann, E., 2011. Alterations in intrinsic membrane properties and the axon initial segment in a mouse model of Angelman syndrome. J. Neurosci. 31, 17637–17648. https://doi.org/10.1523/JNEUROSCI.4162-11.2011.
Kato, C., Tochigi, M., Ohashi, J., Koishi, S., Kawakubo, Y., Yamamoto, K., Matsumoto, H., Hashimoto, O., Kim, S.-Y., Watanabe, K., Kano, Y., Nanba, E., Kato, N., Sasaki, T., 2008. Association study of the 15q11-q13 maternal expression domain in Japanese autistic patients. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 147B, 1008–1012. https://doi.org/10.1002/ajmg.b.30690.
Kim, S.-J., Brune, C.W., Kistner, E.O., Christian, S.L., Courchesne, E.H., Cox, N.J., Cook, E.H., 2008. Transmission disequilibrium testing of the chromosome 15q11-q13 region in autism. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 147B, 1116–1125. https://doi.org/10.1002/ajmg.b.30733.
Kishino, T., Lalande, M., Wagstaff, J., 1997. *UBE3A*/E6-AP mutations cause Angelman syndrome. Nat. Genet. 15, 70–73. https://doi.org/10.1038/ng0197-70.
Krishnan, V., Stoppel, D.C., Nong, Y., Johnson, M.A., Nadler, M.J.S., Ozkaynak, E., Teng, B.L., Nagakura, I., Mohammad, F., Silva, M.A., Peterson, S., Cruz, T.J., Kasper, E.M., Arnaout, R., Anderson, M.P., 2017. Autism gene *UBE3A* and seizures impair sociability by repressing VTA Cbln1. Nature 543, 507–512. https://doi.org/10.1038/nature21678.
Landers, M., Calciano, M.A., Colosi, D., Glatt-Deeley, H., Wagstaff, J., Lalande, M., 2005. Maternal disruption of *UBE3A* leads to increased expression of UBE3A-ATS in trans. Nucleic Acids Res. 33, 3976–3984. https://doi.org/10.1093/nar/gki705.
LaSalle, J.M., Reiter, L.T., Chamberlain, S.J., 2015. Epigenetic regulation of *UBE3A* and roles in human neurodevelopmental disorders. Epigenomics 7. https://doi.org/10.2217/epi.15.70.
Lonsdale, J., Thomas, J., Salvatore, M., Phillips, R., Lo, E., Shad, S., Hasz, R., Walters, G., Garcia, F., Young, N., Foster, B., Moser, M., Karasik, E., Gillard, B., Ramsey, K., Sullivan, S., Bridge, J., Magazine, H., Syron, J., Fleming, J., Siminoff, L., Traino, H., Mosavel, M., Barker, L., Jewell, S., Rohrer, D., Maxim, D., Filkins, D., Harbach, P., Cortadillo, E., Berghuis, B., Turner, L., Hudson, E., Feenstra, K., Sobin, L., Robb, J., Branton, P., Korzeniewski, G., Shive, C., Tabor, D., Qi, L., Groch, K., Nampally, S., Buia, S., Zimmerman, A., Smith, A., Burges, R., Robinson, K., Valentino, K., Bradbury, D., Cosentino, M., Diaz-Mayoral, N., Kennedy, M., Engel, T., Williams, P., Erickson, K., Ardlie, K., Winckler, W., Getz, G., DeLuca, D., MacArthur, D., Kellis, M., Thomson, A., Young, T., Gelfand, E., Donovan, M., Meng, Y., Grant, G., Mash, D., Marcus, Y., Basile, M., Liu, J., Zhu, J., Tu, Z., Cox, N.J., Nicolae, D.L., Gamazon, E.R., Im, H.K., Konkashbaev, A., Pritchard, J., Stevens, M., Flutre, T., Wen, X., Dermitzakis, E.T., Lappalainen, T., Guigo, R., Monlong, J., Sammeth, M., Koller, D., Battle, A., Mostafavi, S., McCarthy, M., Rivas, M., Maller, J., Rusyn, I., Nobel, A., Wright, F., Shabalin, A., Feolo, M., Sharopova, N., Sturcke, A., Paschal, J., Anderson, J.M., Wilder, E.L., Derr, L.K., Green, E.D., Struewing, J.P., Temple, G., Volpi, S., Boyer, J.T., Thomson, E.J., Guyer, M.S., Ng, C., Abdallah, A., Colantuoni, D., Insel, T.R., Koester, S.E., Little, A.R., Bender, P.K., Lehner, T., Yao, Y., Compton, C.C., Vaught, J.B., Sawyer, S., Lockhart, N.C., Demchok, J., Moore, H.F., 2013. The Genotype-Tissue Expression (GTEx) project. Nat. Genet. 45, 580–585. https://doi.org/10.1038/ng.2653 (Expression QTL data retrieved from GTEx Portal database on 23rd of Jan. 2019.).
Lopez, S.J., Segal, D.J., LaSalle, J.M., 2019. *UBE3A*: an E3 ubiquitin ligase with genome-wide impact in neurodevelopmental disease. Front. Mol. Neurosci. 11, 1–8. https://doi.org/10.3389/fnmol.2018.00476.
Mabb, A.M., Judson, M.C., Zylka, M.J., Philpot, B.D., 2011. Angelman syndrome: insights into genomic imprinting and neurodevelopmental phenotypes. Trends Neurosci. 34 (6), 293–303. https://doi.org/10.1016/j.tins.2011.04.001.
Margolis, S.S., Sell, G.L., Zbinden, M.A., Bird, L.M., 2015. Angelman syndrome. Neurotherapeutics 12, 641–650. https://doi.org/10.1007/s13311-015-0361-y.
Mertz, L.G.B., Thaulov, P., Trillingsgaard, A., Christensen, R., Vogel, I., Hertz, J.M., Østergaard, J.R., 2014. Neurodevelopmental outcome in Angelman syndrome: genotype–phenotype correlations. Res. Dev. Disabil. 35, 1742–1747. https://doi.org/10.1016/j.ridd.2014.02.018.
Miao, S., Chen, R., Ye, J., Tan, G.-H., Li, S., Zhang, J., Jiang, Y.-h., Xiong, Z.-Q., 2013. The Angelman syndrome protein *UBE3A* is required for polarized dendrite morphogenesis in pyramidal neurons. J. Neurosci. 33, 327–333. https://doi.org/10.1523/JNEUROSCI.2509-12.2013.
Nicholls, R.D., Knepper, J.L., 2001. Genome organization, function, and imprinting in Prader-Willi and Angelman syndrome. Annu. Rev. Genom. Hum. Genet. 2, 153–175. https://doi.org/10.1146/annurev.genom.2.1.153.
Noor, A., Dupuis, L., Mittal, K., Lionel, A.C., Marshall, C.R., Scherer, S.W., Stockley, T., Vincent, J.B., Mendoza-Londono, R., Stavropoulos, D.J., 2015. 15q11.2 duplication encompassing only the *UBE3A* gene is associated with developmental delay and neuropsychiatric phenotypes. Hum. Mutat. 36, 689–693. https://doi.org/10.1002/humu.22800.
Nurmi, E.L., Amin, T., Olson, L.M., Jacobs, M.M., McCauley, J.L., Lam, A.Y., Organ, E.L., Folstein, S.E., Haines, J.L., Sutcliffe, J.S., 2003. Dense linkage disequilibrium mapping in the 15q11-q13 maternal expression domain yields evidence for association in autism. Mol. Psychiatry 8, 624–634. https://doi.org/10.1038/sj.mp.4001283.
Nurmi, E.L., Bradford, Y., Chen, Y.H., Hall, J., Arnone, B., Gardiner, M.B., Hutcheson, H.B., Gilbert, J.R., Pericak-Vance, M.A., Copeland-Yates, S.A., Michaelis, R.C., Wassink, T.H., Santangelo, S.L., Sheffield, V.C., Piven, J., Folstein, S.E., Haines, J.L., Sutcliffe, J.S., 2001. Linkage disequilibrium at the Angelman syndrome gene *UBE3A* in Autism families. Genomics 77, 105–113. https://doi.org/10.1006/geno.2001.6617.
Peters, S., Beaudet, A., Madduri, N., Bacino, C., 2004. Autism in Angelman syndrome: implications for autism research. Clin. Genet. 66, 530–536. https://doi.org/10.1111/j.1399-0004.2004.00362.x.
Robinson, E.B., St Pourcain, B., Anttila, V., Kosmicki, J.A., Bulik-Sullivan, B., Grove, J., Maller, J., Samocha, K.E., Sanders, S.J., Ripke, S., Martin, J., Hollegaard, M.V., Werge, T., Hougaard, D.M., Neale, B.M., Evans, D.M., Skuse, D., Mortensen, P.B., Børglum, A.D., Ronald, A., Smith, G.D., Daly, M.J., Daly, M.J., 2016. Genetic risk for

autism spectrum disorders and neuropsychiatric variation in the general population. Nat. Genet. 48, 552–555. https://doi.org/10.1038/ng.3529.
Scoles, H.A., Urraca, N., Chadwick, S.W., Reiter, L.T., LaSalle, J.M., 2011. Increased copy number for methylated maternal 15q duplications leads to changes in gene and protein expression in human cortical samples. Mol. Autism 2, 19. https://doi.org/10.1186/2040-2392-2-19.
Sinnema, M., Collin, P., Maaskant, M.A., Van Roozendaal, E.P., Schrander-Stumpel, C.T.R.M., Curfs, L.M.G., 2011a. Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32, 1729–1735. https://doi.org/10.1016/j.ridd.2011.02.027.
Sinnema, M., Einfeld, S.L., Schrander-Stumpel, C.T.R.M., Maaskant, M.A., Boer, H., Curfs, L.M.G., 2011b. Behavioral phenotype in adults with Prader–Willi syndrome. Res. Dev. Disabil. 32, 604–612. https://doi.org/10.1016/j.ridd.2010.12.014.
Smith, S.E.P., Zhou, Y.-D., Zhang, G., Jin, Z., Stoppel, D.C., Anderson, M.P., 2011. Increased gene dosage of *UBE3A* results in autism traits and decreased glutamate synaptic transmission in mice. Sci. Transl. Med. 3, 103ra97. https://doi.org/10.1126/scitranslmed.3002627.
Soni, S., Whittington, J., Holland, A.J., Webb, T., Maina, E., Boer, H., Clarke, D., 2007. The course and outcome of psychiatric illness in people with Prader-Willi syndrome: implications for management and treatment. J. Intellect. Disabil. Res. 51, 32–42. https://doi.org/10.1111/j.1365-2788.2006.00895.x.
Soni, S., Whittington, J., Holland, A.J., Webb, T., Maina, E.N., Boer, H., Clarke, D., 2008. The phenomenology and diagnosis of psychiatric illness in people with Prader–Willi syndrome. Psychol. Med. 38. https://doi.org/10.1017/S0033291707002504.
Stoppel, D.C., Anderson, M.P., 2017. Hypersociability in the Angelman syndrome mouse model. Exp. Neurol. 293, 137–143. https://doi.org/10.1016/j.expneurol.2017.04.002.
Sun, J., Zhu, G., Liu, Y., Standley, S., Ji, A., Tunuguntla, R., Wang, Y., Claus, C., Luo, Y., Baudry, M., Bi, X., 2015. *UBE3A* regulates synaptic plasticity and learning and memory by controlling SK2 channel endocytosis. Cell Rep. 12, 449–461. https://doi.org/10.1016/j.celrep.2015.06.023.
Trillingsgaard, A., Østergaard, J.R., 2004. Autism in Angelman syndrome: an exploration of comorbidity. Autism 8, 163–174. https://doi.org/10.1177/1362361304042720.
van Os, J., Linscott, R.J., Myin-Germeys, I., Delespaul, P., Krabbendam, L., 2009. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. Psychol. Med. 39, 179–195. https://doi.org/10.1017/S0033291708003814.
Vatsa, N., Jana, N.R., 2018. *UBE3A* and its link with autism. Front. Mol. Neurosci. 11, 448. https://doi.org/10.3389/fnmol.2018.00448.
Verhoeven, W.M.A., Tuinier, S., Curfs, L.M.G., 2003. Prader–Willi syndrome: cycloid psychosis in a genetic subtype? Acta Neuropsychiatr. 15, 32–37. https://doi.org/10.1034/j.1601-5215.2003.00006.x.
Vogels, A., Hert, M.De, Descheemaeker, M.J., Govers, V., Devriendt, K., Legius, E., Prinzie, P., Fryns, J.P., 2004. Psychotic disorders in Prader-Willi syndrome. Am. J. Med. Genet. 127A, 238–243. https://doi.org/10.1002/ajmg.a.30004.
Yamamoto, Y., Huibregtse, J.M., Howley, P.M., 1997. The Human E6-AP Gene (*UBE3A*) encodes three potential protein isoforms generated by differential splicing. Genomics 41, 263–266. https://doi.org/10.1006/geno.1997.4617.
Yang, L., Zhan, G., Ding, J., Wang, H., Ma, D., Huang, G., Zhou, W., 2013. Psychiatric illness and intellectual disability in the Prader–Willi syndrome with different molecular defects - a meta analysis. PLoS One 8, e72640. https://doi.org/10.1371/journal.pone.0072640.