Published in final edited form as:

Mech Ageing Dev. 2014 January ; 135: 50–56. doi:10.1016/j.mad.2014.01.005.

Splicing Factor 3B1 hypomethylation is associated with altered *SF3B1* transcript expression in older humans

Alice C. Holly¹, Luke C. Pilling², Dena Hernandez³, Benjamin P. Lee¹, Andrew Singleton³, Luigi Ferrucci⁴, David Melzer², and Lorna W. Harries¹

¹Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, University of Exeter, Exeter, EX2 5DW, UK

²Epidemiology and Public Health, University of Exeter Medical School, University of Exeter, Exeter EX1 2LU, UK

³Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892

⁴National Institute on Aging, Clinical Research Branch, Harbor Hospital, Baltimore, MD 21225

Abstract

Ageing in man is associated with changes to the splicing factor pool. A proportion of splicing factors are regulated during ageing by mechanisms involving the Ataxia Telangiectasia Mutated (*ATM*) gene, but the factors that determine the remaining proportion have yet to be identified.

DNA methylation is known to be an important regulatory mechanism of gene expression. We assessed age-associated methylation and expression levels for 27 splicing factor genes, in peripheral blood samples from the InCHIANTI study. Examination of splicing patterns at specific loci was examined in a second cohort, the Exeter 10000 study.

27/502 methylation probes in 17 different genes were associated with age. Most changes were not associated with transcript expression levels or splicing patterns, but hypomethylation of the *SF3B1* promoter region was found to mediate 53% of the relationship between age and transcript expression at this locus ($p = 0.02$).

DNA methylation does not appear to play a major role in regulation of the splicing factors, but changes in *SF3B1* expression may be attributable to promoter hypomethylation at this locus. *SF3B1* encodes a critical component of the U2 snRNP; altered expression of this gene may therefore contribute to the loss of regulated mRNA splicing that occurs with age.

Keywords

Ageing; splicing; DNA methylation; *SF3B1*; epigenetics

Introduction

Human ageing is characterised by a gradual deterioration and loss of function across all cell types and tissues, and is associated with a gradual decline in tissue repair, genomic stability and stress response. Accordingly, ageing is associated with almost all common chronic diseases in man (Kohn, 1963). These observations have been attributed to multiple mechanisms including impaired DNA damage response, deterioration of the stem cell pool, the Insulin/IGF and mTOR signalling pathways and shortening of telomeres (Kenyon, 2010), however the underlying mechanistic basis for the heterogeneity of human ageing remains poorly understood.

Part of the heterogeneity of ageing and the age-associated increase in common disease may be attributable to loss of fine control of gene expression, by processes such as alternative mRNA splicing (Harries et al., 2011). This is an important co-transcriptional component of gene regulation occurring in most eukaryotic cells, which has been associated with ageing in man (Harries et al., 2011; Holly et al., 2013). This process involves the removal of introns and joining of remaining exons by a number of proteins and regulatory elements and allows fine tuning of gene expression (Cartegni et al., 2002). The majority of human genes undergo alternative mRNA processing, enabling a diverse transcriptome and proteome complexity, enhancing cellular adaptability (Graveley, 2001; Pan et al., 2008). Splicing is regulated by a series of DNA sequence elements that bind the splicing factor machinery. Consensus sequences at the splice donor site, the acceptor site and the branch site are essential for splice site selection, although not usually sufficient (Padgett, 2012); highly regulated or tissue specific splice site usage is also reliant on the binding of auxiliary regulatory elements such as serine/arginine-rich splicing enhancers (SRSFs) and heterogeneous nuclear ribonucleoproteins (hnRNPs). These regulatory elements bind regions termed intronic and exonic splicing enhancers and silencers (ESEs/ISEs, ISSs/ESSs) (Cartegni et al., 2002), and act to promote or inhibit splicing of a particular exon (Cartegni et al., 2002; Smith and Valcárcel, 2000). Our previous work has demonstrated that changes in the splicing factor milieu are associated with both human ageing *in vivo*, and *in vitro* cellular senescence (Harries et al., 2011; Holly et al., 2013). Using microarray data from peripheral blood samples from the InCHIANTI population (Ferrucci et al., 2000), we identified age-associated changes in transcript expression for 27 of 74 splicing factor genes examined in our previous study (Holly et al., 2013). Similar changes have also been reported in various tissues in cell systems and in animal models of ageing (Cao et al., 2001; Lee et al., 1999; Shelton et al., 1999; Tollet-Egnell et al., 2001) indicating that this may be a global process and therefore may have profound implications for cellular functioning and cellular senescence. Our data suggests that a proportion of the splicing factor pool may be regulated by mechanisms involving the DNA damage response protein Ataxia Telangiectasia Mutated (ATM) (Holly et al., 2013), but no potential reason for the age-related decrease in the expression of the remaining splicing factors has yet been determined.

The importance of epigenetic changes such as DNA and histone modifications in the regulation of gene expression is now becoming evident (Fuks, 2005). DNA methylation has a major role in tissue specificity and regulation of gene expression (Aran et al., 2011; Lyko et al., 2010; Meissner et al., 2008). Methylated Cytosine bases occur at Cytosine-Guanine

dinucleotide (CpG) sites, which are present mainly in regions termed CpG islands (CGIs). Over two thirds of promoter regions contain CpG islands (Rollins et al., 2006) (Saxonov et al., 2006) and the majority of promoter sites remain unmethylated (Bird, 2002). Methylation at a promoter site is usually associated with decreases in gene expression and mechanisms of gene repression either by preventing the binding of transcription factors or recruiting methyl binding proteins which promote inactive chromatin (Boyes and Bird, 1991; Geiman and Robertson, 2002; Jones and Baylin, 2007). It is also emerging that DNA methylation may also play a prominent role in regulating splicing events; the percentage of methylated CpG residues are more prevalent in exons relative to flanking introns (Laurent et al., 2010) and alternative splice sites have a lower level of methylation than constitutive splice sites (Gelfman et al., 2013). Splicing and transcription are simultaneous processes; nascent RNA transcripts are therefore spliced whilst still attached to the DNA template. For this reason, DNA methylation may also moderate splicing. Alterations to DNA methylation has previously been noted in human ageing, and also in association with lifestyle and environment (Feil and Fraga, 2011; Fraga et al., 2005). DNA methylation during ageing commonly features hypomethylation at promoter, exonic, intragenic and intronic regions, whilst hypermethylation is usually noted CpG rich promoter regions (Johansson et al., 2013). DNA methylation is highly tissue specific but human lymphocytes have been shown to have a global decrease in DNA methylation with age (Christensen et al., 2009; Fuke et al., 2004a).

This raises the possibility that some of the observed differences in the composition of the pool of splicing regulator transcripts with age could arise from changes in the DNA methylation levels of the splicing factor genes themselves. Like 95% of all human genes, the genes responsible for regulation of the splicing process are also alternatively spliced. Methylation differences with age could therefore alter either the total expression level of the gene in question, or alter the balance of splice forms produced. To address this possibility we correlated methylation patterns with age, for a subset of 27 splicing factor genes, with known effects of age on transcript expression. This analysis was carried out using data from a cross-sectional population, the InCHIANTI study (Holly et al., 2013). Where age-related differences in DNA methylation levels were present, the effect of altered DNA methylation on transcript expression was examined in the InCHIANTI data by linear regression and mediation models. Finally, where the location of differentially methylated regions suggested a potential role in moderation of splicing, we assessed the relationship between the expression of specific isoforms and age in a second cohort, the Exeter 10000 study. By these means, we hope to determine whether age-associated changes to the splicing factor milieu could be attributable to altered DNA methylation in aged cells.

Methods

Study populations

The InCHIANTI population is a large population-based study based in the Chianti region of Tuscany, Italy (Ferrucci et al., 2000). Participants have been followed up on 3 occasions between 2000 and 2009 since the start of the study. Ethical approval was granted by the Istituto Nazionale Riposo e Cura Anziani institutional review board. Samples for DNA and

RNA analysis for this analysis were collected at the same timepoint in the third wave of the study in 2008/2009. Genomic DNA was extracted from buffy coat samples on the AutoGen Flex system (Autogen, USA) and quantified on a Nanodrop1000 spectrophotometer and RNA was extracted from whole blood using the PAXgene system. Although DNA was available at each wave, RNA was only available at the third timepoint. 506 participants had data for both expression and methylation following QC, and all of these were included in our study.

The Exeter 10000 study cohort is healthy subjects from the South West of the UK. The individuals included in the cohort were 53% female and 47% male and were of predominantly White British origin. The age range was from 16 years to 87 years (mean 54.7, SD 14.2). Whole blood samples were collected in 2011/2012 using the PAXgene system (Debey-Pascher et al., 2009) for RNA analysis. We have selected individuals available with the largest age range for analysis. Ethical approval was granted by the Bristol Regional Ethics Committee (study number 09/H0106/75). Written informed consent was obtained for all participants.

Selection of splicing factors for study

A list of known splicing factors was identified from the SpliceFac database (<http://rulai.cshl.edu/cgi-bin/SpliceFac/spliceFac.cgi?process=home>). 72 splicing factors were expressed in whole blood, 27 of which displayed age-associated changes in their expression in our previous work (Harries et al., 2011; Holly et al., 2013) (See supplementary table 1). These splicing factors were selected for inclusion in this analysis.

Generation of Methylation data

Genomic DNA was bisulfite converted using Zymo EZ-96 DNA Methylation Kit per the manufacturer's protocol. CpG methylation status of 485,577 CpG sites was determined using Illumina Infinium HumanMethylation450 BeadChip per manufacturer's protocol and as previously described (Gibbs et al., 2010). Initial data analysis was performed using GenomeStudio 2011.1 (Model M Version 1.9.0 Illumina, Inc. CA)). Threshold call rate for inclusion of samples was 95%. Quality control of sample handling included comparison of clinically reported sex versus sex of the same samples determined by analysis of methylation levels of CpG sites on the X chromosome. Beta values were extracted for sites on the X chromosome. Subject mean methylation versus subject mean intensity levels were plotted in R V2.11.1. Based on methylation levels for chromosome X loci, these data split into two primary groups. Calls generated by this method were then compared with sample information reported by InCHIANTI. Samples not matching between clinical reported sex and methylation data were excluded from analyses. 5 samples were excluded on the basis of sex-mismatch, 8 samples were omitted on the basis of other QC criteria.

Quality Control of methylation data

Quantile normalization of the methylation arrays was carried out using package "wateRmelon" for the R statistical computing language (Pidsley et al., 2013). The DASEN method was applied, which performs the quantile normalization separately on M and U (methylated/un-methylated) values, and also separates the type 1 and type 2 Infinium probes.

This minimises the technical variance between the arrays, whilst taking into consideration the different technologies present on the arrays. 1 sample failed QC, leaving methylation data for 506 InCHIANTI participants following quality control and data cleaning.

Both type 1 and type 2 probes were included in our analysis. Methylation probes were assigned to genes according to the Bioconductor repository package (<http://www.bioconductor.org/packages/2.13/data/annotation/html/IlluminaHumanMethylation450k.db.html>). All methylation signals annotated to a specific gene were included in our analysis unless they were excluded on the basis of non-specificity (Chen et al., 2013) or had been reported to overlap a single nucleotide polymorphism (SNP) with a minor allele frequency of greater than 0.5% in 1000 genomes integrated phase 1 data (<http://www.1000genomes.org/ftp://ftptrace.ncbi.nih.gov/1000genomes/ftp/release/20110521/>) (supplementary tables 2 and 3). Probes located to CpG islands, shores and shelves throughout the targeted genes.

Assessment of potential correlations between DNA methylation and age for 27 transcripts coding for splicing regulatory proteins

Methylation probes binding within the 27 splicing factor genes where mRNA expression levels were known to be associated with age (see supplementary table 1) were identified from the Illumina annotation of the methylation chip data. All other methylation probes located in the 27 genes of interest that presented a signal following quality control measures were included in our analysis. Associations between age and DNA methylation level at these loci was assessed by fully adjusted multiple linear regression models (R statistical package v.3) with methylation signal as the outcome variable. Models were adjusted for confounding factors including batch effects, sex, waist circumference (continuous trait), smoking status in lifetime pack years smoked (in five categories: none, <20yrs, 20–39 years, 40+ yrs) education level in five categories (none, elementary, secondary, high school, and university), study site and white cell subtype count (neutrophil, lymphocyte, monocyte and eosinophil percentages). Multiple testing was accounted for by Bonferroni correction.

Correlation of methylation level and transcript expression for splicing factor genes

For each of the 27 splicing factor genes we assessed the association of each methylation probe on expression individually using linear regression models, adjusted for the same confounding factors as in the assessment of methylation and age. Each of these models was performed twice, once with and once without age as a covariate, so that the effect of adjusting for age could be assessed.

Potential mediation of transcript expression levels by differential DNA methylation

The subset of methylation probes that remained associated with age following Bonferroni correction for multiple testing were then examined to determine how much of the association between age and splicing factor transcript expression could potentially be mediated by age-related methylation differences. A linear multiple regression model was applied, with splicing factor transcript expression as the outcome and age as the independent variable. Methylation signal was included with other standard confounding variables, as described above.

A second linear multiple regression model which was not adjusted for the methylation signal was then undertaken, and the percentage mediation could then be calculated from the difference between the coefficients for the association between age and expression, with and without accounting for the effect of methylation. In order to determine the statistical significance of the mediating relationships we applied the Sobel mediation test (Sobel, 1982) which derives a t-statistic from the standard errors of the models described in the previous section. A corresponding p value can then be calculated from the normal distribution.

Design of probes to detect alternatively spliced gene transcripts

Altered methylation could alter the output from the splicing factor genes by modulation of the expression ratio of alternatively expressed transcripts, which may not manifest as a difference in total expression. Where age-associated methylation probes were located in regions with potential to affect splice site choice, we designed isoform-specific probes to assess this possibility. In order for genes to be included in transcript analysis, the age-associated methylation probe had to be located within alternatively spliced regions of known coding isoforms of the splicing factors in question. Transcript sequences of alternatively expressed isoforms were identified and aligned using the UCSC web interface (<http://genome.ucsc.edu/>), and probes were designed to unique regions of the alternatively spliced transcripts, crossing exon boundaries wherever possible to ensure amplification of sequences deriving from mRNA rather than hnRNA or contaminating genomic DNA. Probe and primer sequences for alternatively spliced isoforms of *SRSF1* and *HNRNPA1* are given in supplementary table 4.

RNA collection, extraction and analysis of alternative splicing of the splicing factor genes

We assessed splice site usage with age for two genes fitting criteria for splicing analysis (see above), *SRSF1* and *HNRNPA1*. We quantified the abundance of each alternatively processed transcript of these two genes in 53 young (<60 years) and 43 older (≥ 60) participants from the Exeter 10000 study. The PAXgene Blood mRNA kit (Qiagen, Crawley, UK) was used to extract RNA from peripheral blood samples according to the manufacturer's instructions. 100ng of total RNA was reverse transcribed using Superscript III VILO kit (Invitrogen, Paisley, UK) in 20µl reactions. We measured the expression levels of each alternatively spliced transcript by qRT-PCR carried out on each sample in triplicate in 96 well plates on the ABI Prism 7900HT platform (Life Technologies, Foster City, USA). Each reaction contained 5µl TaqMan Universal Mastermix (no AMPerase) (Life Technologies, Foster City USA), 0.25 µM probe and 1µl cDNA reverse transcribed as above in a total volume of 10µl. PCR conditions were one cycle of 50°C for 2 minutes, one cycle of 95°C for 10 minutes followed by 60 cycles of 95°C for 15 seconds and 60°C for 1 minute. The expression levels of alternatively spliced transcripts were determined by the comparative Ct method (Pfaffl, 2001), expressed relative to the *B2M* (NM_004048.2) and *PPIA* (NM_021130.3) endogenous control genes, which were known not to be associated with age. The results were expressed as a ratio of alternatively spliced transcript expression and normalized back to the ratio of the transcript in question in younger subjects and the ratios of the probes determined relative to age. The difference between the ratio of isoforms expressed in young and older individuals was assessed by Mann Whitney-U analysis (SPSS V20, IBM).

RESULTS

Evidence for 25 age-associated regions of differential DNA methylation in splicing factor genes

We assessed the methylation patterns of 27 splicing factor genes for which we had previously demonstrated associations with age in man (Harries et al., 2012; Holly et al., 2013). 543 methylation probes were found to locate near to genes coding for the splicing factors in question, 5 of these were excluded as the minor allele frequency was reported as greater than 0.5% and a further 36 methylation probes were excluded due to non-specific binding (Supplementary table 2 and 3). Of the remaining 502 methylation probes we found that 27 probes located within 17 different genes were significantly associated with age after Bonferroni correction for multiple testing ($<9.96 \times 10^{-5}$) (table 1). The top three age-related methylation sites in our study were cg23960707 (*SRSF1*), cg08591265 (*SRSF1*) and cg22718189 (*SRSF11*) (Beta coefficients = -0.001 , <-0.001 and <-0.001 ; p-values = 9.05×10^{-17} , 1.43×10^{-14} and 4.26×10^{-11} respectively). Among the Bonferroni significant CpG sites studied, 12 are in gene body regions, 4 in first exon/5' untranslated region, 9 within 155bp of the transcription start site, and two in a 3' untranslated region (supplementary table 1).

DNA methylation and expression levels are correlated only for *SF3B1*

We assessed the degree of association between the expression and methylation signal for 27 DNA methylation probes within 17 genes that demonstrated an association with age. Of the 27 methylation probes tested only two demonstrated correlations between expression and DNA methylation levels. Probes cg07204893 and cg25839482 located in the promoter regions of the *SF3B1* and *IMP3* genes respectively, demonstrated statistically significant associations between methylation level and expression of the gene in question; coefficient = 154.76, 95% CI = 36.52 to 273; p = 0.01 and coefficient = -219.19 , 95% CI = -405.48 to -32.90 , for *SF3B1* and *IMP3* respectively (table 2). The remaining 25 methylation probes demonstrated no statistically significant associations between expression of the respective transcripts and methylation levels (table 2).

DNA methylation is a potential modifier of *SF3B1* transcript expression

We next sought to determine the degree to which epigenetic changes within the DNA sequence of the *SF3B1* and *IMP3* genes influenced transcript expression level, by comparison of the size of the beta coefficient for association with and without inclusion of methylation status as a cofounder. We were able to determine that DNA methylation at cg07204893 mediates approximately 53% of the association between *SF3B1* transcript expression and age (Sobel p value = 0.02). The position of the methylation probe cg07204893 within the *SF3B1* gene is given in figure 1a. Although DNA methylation changes at cg25839482 has the potential to mediate 8% of the association between *IMP3* transcript expression and age, this result is not statistically significant (Sobel p value = 0.19).

The *SRSF1* and *HNRNPA1* genes contain age-associated regions of differential methylation that have potential to alter splice site usage

DNA methylation changes could alter the composition of the splicing factor milieu by altering total gene expression, or by modulating the splicing of the splicing factors themselves. This would manifest as a change to the nature, rather than the amount of splicing factor transcripts expressed. We found that alternatively spliced regions of two genes; *SRSF1* and *HNRNPA1*, contained age-associated regions of differential methylation. The 2 most age-associated DNA methylation signals, cg23960707 (figure 1b; probe B) and cg08591265 (figure 1b; probe C), were both located within an alternatively spliced region of the *SRSF1* gene which was distinct from the CpG island (beta coefficients -0.001 and -0.002 ; p values 1.391×10^{-19} and 2.192×10^{-12} respectively) (see figure 1b). An additional methylation site identified by cg21197224 (figure 1b; probe D) was located within *SRSF1* exon 4 and is associated with age after correction for multiple testing (beta coefficient < -0.001 , p value 1.245×10^{-7}). *SRSF1* produces two coding splice variants. Isoform 1 (NM_006924.4), containing exon 4, is the longest transcript and isoform 2 (NM_001078166.1) which uses an alternative splice donor site at intron 3, and introduces a shortened C-terminus. We were able to design an assay specific to isoform 1, which allowed us to quantify the ratio of isoform 1 relative to the expression of isoform 1 and isoform 2 combined.

An age-associated area of differential DNA methylation (beta coefficient -0.001 ; p value 0.001) was identified by probe cg02583484 within the *HNRNPA1* gene (figure 1c, probe E; p value = <0.001 , B <-0.001). This probe is situated in the region spanning the splice donor site of intron 8. The *HNRNPA1* gene has two known coding alternative splice variants (figure 1c); isoform 1 and isoform 2. Isoform 1 (NM_002136.2) is the shorter transcript with one less in-frame exon than isoform 2 (NM_031157.2). We were able to design assays specific to each isoform, allowing quantification of isoform 1 relative to isoform 2.

SRSF1 and *HNRNPA1* exon usage does not alter with age

We assessed the splice site usage of two splicing factor genes, *SRSF1* and *HNRNPA1*, where age-associated regions of differential methylation were located in or near alternatively spliced exons. We found no evidence to support age-related differences in the ratio of alternatively expressed isoforms for either *SRSF1* or *HNRNPA1*. The ratio of isoforms was not significantly different in young or aged subjects for either gene. For *SRSF1*, the median transcript expression was 1.00, [IQR 0.27] compared with 0.97 [IQR = 0.27] in the older subjects (p = 0.997). For *HNRNPA1*, the median ratio of isoforms in the young was 0.83 [IQR 1.68] compared with a median of 1.87 [IQR 1.90] in the older subjects (p = 0.441, figures 2a and 2b). Although this difference in median may appear considerable, this is due to the large spread of data and the inter-individual variability in *HNRNPA1* isoform ratio.

Discussion

Changes in the regulation of mRNA splicing are known to occur with age (Harries et al., 2011; Holly et al., 2013), and may form a major driving force for ageing and age related disease. We have previously identified that approximately one third of the splicing factors

responsible for moderation of the splicing process may be regulated by the DNA damage response protein ATM (Holly et al., 2013), but details of the control of the remaining splicing factors remains elusive. In this study, we sought to determine whether changes in the composition of the available splicing factor pool could be influenced by age-related changes in DNA methylation at the genes coding for these factors.

We have identified that alterations of DNA methylation patterns with age are present in 17/27 (63%) of splicing factors that have age-associated expression patterns. The majority of these have decreased DNA methylation with age (hypomethylation), which is consistent with the published literature (Fuke et al., 2004b). Our data indicate that hypomethylation of a region in close proximity to the promoter of the *SF3B1* gene appears to be a strong regulator of *SF3B1* transcript expression, with 53% of the age-related down-regulation in the expression of the *SF3B1* gene potentially due to DNA methylation changes at this locus. Reduced DNA methylation is usually associated with open chromatin structure and the presence of active regulatory elements. Transcriptional control has both positive and negative elements, with enhancers serving to promote gene expression but insulators serving to inhibit gene expression (Sigrist and Pirrotta, 1997; West et al., 2002). Accordingly, the differentially methylated residue identified by the chip resides in a region of open chromatin, indicated by acetylation at the K27 position of histone 3 (H3K27Ac) with evidence of chromatin modifications in multiple cell lines in publically available ENCODE data accessed through the UCSC web interface (<http://genome.ucsc.edu/>) (see figure 3). This may indicate a potential binding site for a negative regulatory factor, which may silence *SF3B1* expression.

SF3B1 encodes a crucial constituent of the U2 snRNP. This is a key component of the spliceosome, the collection of nucleic acids and proteins that co-ordinates and executes the splicing process. The U2 snRNP is the point of interaction between the spliceosome and the splice site branch point and represents an essential step in the formation of lariat structures required for liberation of the intron during splicing. Reduction of the availability of the U2 snRNP would be expected to affect all splicing events in the cell. We predict that a decrease in the availability of *SF3B1* transcripts during the ageing process may affect the available pool of U2snRNPs, potentially leading to global deregulation of splicing. Cellular senescence is governed by many of the same genes that determine proliferative capacity such as *TP53* and *CDKN2A* (Pospelova et al., 2013), and many senescence genes are also proto-oncogenes. Accordingly, *SF3B1* mutations have been associated with several tumour types including uveal melanoma (Furney et al., 2013), adenoid cystic carcinoma (Stephens et al., 2013), but seem to play a particular role in several subtypes of leukaemia and in myelodysplastic syndromes (Quesada et al., 2012; Rossi et al., 2011). Expression and methylation levels for the remaining splicing factors displayed little correlation, indicating that the altered DNA methylation may not cause effects at the transcriptional level for the other splicing factors.

There is increasing evidence that DNA methylation could also moderate the mRNA splicing process (Lyko et al., 2010; Shukla et al., 2011). The genes coding for the splicing factors themselves are also alternatively spliced, which is a potential mechanism for the regulation of gene activity. Changes in DNA methylation may thus affect the nature, rather than the

amount of transcript produced. We identified two age-associated regions of differential methylation in the *SRSF1* and *HNRNPA1* genes, which were located in regions of known alternative splicing. Three regions identified by probes cg23960707 cg08591265 and cg21197224 were located within alternatively expressed exons of the *SRSF1* (SF2/ASF) gene. SRSF1 is an important splicing regulatory factor which binds ESEs to enhance splice site usage (Cartegni et al., 2002). Similarly, one differentially methylated region, identified by probe cg02583484, was located across the alternative splice junction of exon 8 within the *HNRNPA1* gene. Despite promising bioinformatic data suggesting the presence of bound RNA polymerase II and CCTCF (see supplementary figure 1a and 1b.), both markers of ribosome pausing and alternative splicing (Foret et al., 2012; Ip et al., 2011; Wan et al., 2013), we found no evidence to support altered *SRSF1* or *HNRNPA1* splicing with age, suggesting that the regions of differential methylation may not mediate alternative splicing at these loci.

The strengths of our study are the use of both mRNA expression and DNA methylation data collected at the same time point from a large cross-sectional population of well-characterised people. The work continues and builds on our previous analyses of splicing regulation with age, and is amongst the first to address the possibility of age-related changes to the amount or nature of the splicing factor pool arising from altered DNA methylation. The limitations of our study include the analysis of mRNA, rather than protein levels, but mRNA levels are a reasonable proxy for demand for specific splicing factors in the cells and the use of mRNA screens is well established. DNA methylation is also tissue and cell type specific (Day et al., 2013) and our results will thus be representative of the tissue type we have analysed – peripheral blood leukocytes. However, blood is the only viable option for high throughput study of in-vivo expression and methylation in humans, which would not be feasible in other tissue types. It is possible the association between *SF3B1* expression and DNA methylation at this locus may not be evident in all tissue types, but the U2 snRNP is essential for splicing activity (Query et al., 1994), and as such, should be represented in all tissue types. A possible limitation of the study is that a number of the methylation probes have been identified as non-specific, or affected by common single-nucleotide polymorphisms (SNPs) (Chen et al., 2013). To reduce the possibility that our results are affected we excluded probes reported as having any cross-reactivity, and excluded others that have a SNP underlying the probe binding site with a minor allele frequency greater than 0.5%.

To conclude, we have identified age-associated alterations to DNA methylation patterns in 17 of 27 splicing factors known to show changes to transcript expression during ageing in man. In particular, hypomethylation within the promoter region of one splicing factor, *SF3B1*, appears to be a major regulator of *SF3B1* transcript levels, mediating 53% of its expression. *SF3B1* encodes a critical component of the U2 snRNP, which is essential for branch site recognition during the splicing process. Altered *SF3B1* expression is also evident in several cancers. Decreased expression of such a core spliceosomal component may therefore be a contributor to the changes to regulated mRNA splicing that occurs during human ageing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Aran D, Toperoff G, Rosenberg M, Hellman A. Replication timing-related and gene body-specific methylation of active human genes. *Human Molecular Genetics*. 2011; 20:670–680. [PubMed: 21112978]
- Bird A. DNA methylation patterns and epigenetic memory. *Genes & Development*. 2002; 16:6–21. [PubMed: 11782440]
- Boyes J, Bird A. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell*. 1991; 64:1123–1134. [PubMed: 2004419]
- Cao SX, Dhahbi JM, Mote PL, Spindler SR. Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice. *Proceedings of the National Academy of Sciences*. 2001; 98:10630–10635.
- Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet*. 2002; 3:285–298. [PubMed: 11967553]
- Chen, Y-a, Lemire, M., Choufani, S., Butcher, DT., Grafodatskaya, D., Zanke, BW., Gallinger, S., Hudson, TJ., Weksberg, R. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics*. 2013; 8:0–1.
- Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Padbury JF, Bueno R, Sugarbaker DJ, Yeh RF, Wiencke JK, Kelsey KT. Aging and Environmental Exposures Alter Tissue-Specific DNA Methylation Dependent upon CpG Island Context. *PLoS Genet*. 2009; 5:e1000602. [PubMed: 19680444]
- Day K, Waite LL, Thalacker-Mercer A, West A, Bamman MM, Brooks JD, Myers RM, Absher D. Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. *Genome Biol*. 2013; 14:R102. [PubMed: 24034465]
- Debey-Pascher S, Eggle D, Schultze JL. RNA stabilization of peripheral blood and profiling by bead chip analysis. *Methods Mol Biol*. 2009; 496:175–210. [PubMed: 18839112]
- Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet*. 2011; 13:97–109.
- Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, Guralnik JM. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc*. 2000; 48:1618–1625. [PubMed: 11129752]
- Foret S, Kucharski R, Pellegrini M, Feng S, Jacobsen SE, Robinson GE, Maleszka R. DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. *Proc Natl Acad Sci U S A*. 2012; 109:4968–4973. [PubMed: 22416128]
- Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A*. 2005; 102:10604–10609. [PubMed: 16009939]
- Fuke C, Shimabukuro M, Petronis A, Sugimoto J, Oda T, Miura K, Miyazaki T, Ogura C, Okazaki Y, Jinno Y. Age related changes in 5-methylcytosine content in human peripheral leukocytes and placentas: an HPLC-based study. *Ann Hum Genet*. 2004a; 68:196–204. [PubMed: 15180700]
- Fuke C, Shimabukuro M, Petronis A, Sugimoto J, Oda T, Miura K, Miyazaki T, Ogura C, Okazaki Y, Jinno Y. Age Related Changes in 5-methylcytosine Content in Human Peripheral Leukocytes and Placentas: an HPLC-based Study. *Annals of Human Genetics*. 2004b; 68:196–204. [PubMed: 15180700]
- Fuks F. DNA methylation and histone modifications: teaming up to silence genes. *Current Opinion in Genetics & Development*. 2005; 15:490–495. [PubMed: 16098738]

- Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, Desjardins L, Turajlic S, Piperno-Neumann S, de la Grange P, Roman-Roman S, Stern MH, Marais R. SF3B1 mutations are associated with alternative splicing in uveal melanoma. *Cancer Discov.* 2013
- Geiman TM, Robertson KD. Chromatin remodeling, histone modifications, and DNA methylation—how does it all fit together? *Journal of Cellular Biochemistry.* 2002; 87:117–125. [PubMed: 12244565]
- Gelfman S, Cohen N, Yearim A, Ast G. DNA-methylation effect on cotranscriptional splicing is dependent on GC architecture of the exon–intron structure. *Genome Research.* 2013; 23:789–799. [PubMed: 23502848]
- Graveley BR. Alternative splicing: increasing diversity in the proteomic world. *Trends in Genetics.* 2001; 17:100–107. [PubMed: 11173120]
- Harries LW, Fellows AD, Pilling LC, Hernandez D, Singleton A, Bandinelli S, Guralnik J, Powell J, Ferrucci L, Melzer D. Advancing age is associated with gene expression changes resembling mTOR inhibition: evidence from two human populations. *Mech Ageing Dev.* 2012; 133:556–562. [PubMed: 22813852]
- Harries LW, Hernandez D, Henley W, Wood AR, Holly AC, Bradley-Smith RM, Yaghootkar H, Dutta A, Murray A, Frayling TM, Guralnik JM, Bandinelli S, Singleton A, Ferrucci L, Melzer D. Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. *Aging Cell.* 2011; 10:868–878. [PubMed: 21668623]
- Holly AC, Melzer D, Pilling LC, Fellows AC, Tanaka T, Ferrucci L, Harries LW. Changes in splicing factor expression are associated with advancing age in man. *Mech Ageing Dev.* 2013
- Ip JY, Schmidt D, Pan Q, Ramani AK, Fraser AG, Odom DT, Blencowe BJ. Global impact of RNA polymerase II elongation inhibition on alternative splicing regulation. *Genome Res.* 2011; 21:390–401. [PubMed: 21163941]
- Johansson Å, Enroth S, Gyllenstein U. Continuous Aging of the Human DNA Methylome Throughout the Human Lifespan. *PLoS ONE.* 2013; 8:e67378. [PubMed: 23826282]
- Jones PA, Baylin SB. The Epigenomics of Cancer. *Cell.* 2007; 128:683–692. [PubMed: 17320506]
- Kenyon CJ. The genetics of ageing. *Nature.* 2010; 464:504–512. [PubMed: 20336132]
- Kohn RR. Human aging and disease. *Journal of Chronic Diseases.* 1963; 16:5–21. [PubMed: 14034238]
- Laurent L, Wong E, Li G, Huynh T, Tsigos A, Ong CT, Low HM, Kin Sung KW, Rigoutsos I, Loring J, Wei CL. Dynamic changes in the human methylome during differentiation. *Genome Research.* 2010; 20:320–331. [PubMed: 20133333]
- Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene Expression Profile of Aging and Its Retardation by Caloric Restriction. *Science.* 1999; 285:1390–1393. [PubMed: 10464095]
- Lyko F, Foret S, Kucharski R, Wolf S, Falckenhayn C, Maleszka R. The Honey Bee Epigenomes: Differential Methylation of Brain DNA in Queens and Workers. *PLoS Biol.* 2010; 8:e1000506. [PubMed: 21072239]
- Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, Lander ES. Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature.* 2008; 454:766–770. [PubMed: 18600261]
- Padgett RA. New connections between splicing and human disease. *Trends in Genetics.* 2012; 28:147–154. [PubMed: 22397991]
- Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet.* 2008; 40:1413–1415. [PubMed: 18978789]
- Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 2001; 29:e45. [PubMed: 11328886]
- Pidsley R, CCYW, Volta M, Lunnon K, Mill J, Schalkwyk LC. A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics.* 2013; 14:293. [PubMed: 23631413]
- Pospelova TV, Chitikova ZV, Pospelov VA. An integrated approach for monitoring cell senescence. *Methods Mol Biol.* 2013; 965:383–408. [PubMed: 23296673]

- Query CC, Moore MJ, Sharp PA. Branch nucleophile selection in pre-mRNA splicing: evidence for the bulged duplex model. *Genes Dev.* 1994; 8:587–597. [PubMed: 7926752]
- Quesada V, Conde L, Villamor N, Ordonez GR, Jares P, Bassaganyas L, Ramsay AJ, Bea S, Pinyol M, Martinez-Trillos A, Lopez-Guerra M, Colomer D, Navarro A, Baumann T, Aymerich M, Rozman M, Delgado J, Gine E, Hernandez JM, Gonzalez-Diaz M, Puente DA, Velasco G, Freije JM, Tubio JM, Royo R, Gelpi JL, Orozco M, Pisano DG, Zamora J, Vazquez M, Valencia A, Himmelbauer H, Bayes M, Heath S, Gut M, Gut I, Estivill X, Lopez-Guillermo A, Puente XS, Campo E, Lopez-Otin C. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet.* 2012; 44:47–52.
- Rollins RA, Haghghi F, Edwards JR, Das R, Zhang MQ, Ju J, Bestor TH. Large-scale structure of genomic methylation patterns. *Genome Research.* 2006; 16:157–163. [PubMed: 16365381]
- Rossi D, Brusca A, Spina V, Rasi S, Khiabani H, Messina M, Fangazio M, Vaisitti T, Monti S, Chiaretti S, Guarini A, Del Giudice I, Cerri M, Cresta S, Deambrogi C, Gargiulo E, Gattei V, Forconi F, Bertoni F, Deaglio S, Rabadan R, Pasqualucci L, Foà R, Dalla-Favera R, Gaidano G. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. *Blood.* 2011; 118:6904–6908. [PubMed: 22039264]
- Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103:1412–1417. [PubMed: 16432200]
- Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. *Current Biology.* 1999; 9:939–945. [PubMed: 10508581]
- Shukla S, Kavak E, Gregory M, Imashimizu M, Shutinoski B, Kashlev M, Oberdoerffer P, Sandberg R, Oberdoerffer S. CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature.* 2011; 479:74–79. [PubMed: 21964334]
- Sigrist CJ, Pirrotta V. Chromatin insulator elements block the silencing of a target gene by the *Drosophila* polycomb response element (PRE) but allow trans interactions between PREs on different chromosomes. *Genetics.* 1997; 147:209–221. [PubMed: 9286681]
- Smith CWJ, Valcárcel J. Alternative pre-mRNA splicing: the logic of combinatorial control. *Trends in Biochemical Sciences.* 2000; 25:381–388. [PubMed: 10916158]
- Sobel M. Asymptotic Confidence Intervals for Indirect Effects in Structural Equation Models. *Sociological Methodology.* 1982; 13:290–312.
- Stephens PJ, Davies HR, Mitani Y, Van Loo P, Shlien A, Tarpey PS, Papaemmanuil E, Cheverton A, Bignell GR, Butler AP, Gamble J, Gamble S, Hardy C, Hinton J, Jia M, Jayakumar A, Jones D, Latimer C, McLaren S, McBride DJ, Menzies A, Mudie L, Maddison M, Raine K, Nik-Zainal S, O'Meara S, Teague JW, Varela I, Wedge DC, Whitmore I, Lippman SM, McDermott U, Stratton MR, Campbell PJ, El-Naggar AK, Futreal PA. Whole exome sequencing of adenoid cystic carcinoma. *J Clin Invest.* 2013; 123:2965–2968. [PubMed: 23778141]
- Tollet-Egnell P, Flores-Morales A, Ståhlberg N, Malek RL, Lee N, Norstedt G. Gene Expression Profile of the Aging Process in Rat Liver: Normalizing Effects of Growth Hormone Replacement. *Molecular Endocrinology.* 2001; 15:308–318. [PubMed: 11158336]
- Wan J, Oliver VF, Zhu H, Zack DJ, Qian J, Merbs SL. Integrative analysis of tissue-specific methylation and alternative splicing identifies conserved transcription factor binding motifs. *Nucleic Acids Res.* 2013
- West AG, Gaszner M, Felsenfeld G. Insulators: many functions, many mechanisms. *Genes Dev.* 2002; 16:271–288. [PubMed: 11825869]

Figure 1. The exon structure and probe placement for the *SF3B1*, *SRSF1* and *HNRNPA1* genes
The structure of transcripts of the *SF3B1*, *SRSF1* and *HNRNPA1* genes are given along with the position of the age-related DNA methylation probes relative to the isoform structure. Slim grey boxes refer to non-translated regions, thick boxes refer to coding exons. Black bars indicate methylation probes. A – cg07204893, B – cg23960707, C – cg08591265, D – cg21197224 and E – cg02583484.

Figure 2. Box plots showing the ratio of alternatively expressed isoforms of the *SRSF1* (A) and *HNRNPA1* (B) genes in relation to age

The ratios of the standardised gene expressions relative to endogenous controls are given on the Y-axis and the population age groups on the X-axis, where young ≤ 60 and old > 60 .

Figure 3. Histone modification and chromatin accessibility in the region of the *SF3B1* promoter
 The gene structure, nature of the chromatin and the presence of activating histone marks in the region of the *SF3B1* promoter as determined from ENCODE data available in the UCSC Genome Browser (<http://genome.ucsc.edu/>) are given. The site of differential DNA methylation identified by cg07204893 is indicated by the light grey bar.

Table 1
Correlation of DNA methylation with age for a subset of splicing factor genes

CpG methylation probes which are associated with age in human blood samples from the InCHIANTI cohort (n=506) after Bonferroni correction for multiple testing.. The gene and probe identities are given along with the coefficients, 95% confidence intervals and p values are given.

ProbeID	Gene	P value	Coefficient	LCI	UCI
cg23960707	SRSF1	9.05×10^{-17}	-1.36×10^{-3}	-1.67×10^{-3}	-1.06×10^{-3}
cg08591265	SRSF1	3.77×10^{-12}	-1.87×10^{-3}	-2.39×10^{-3}	-1.36×10^{-3}
cg22718189	SFRS11	4.26×10^{-11}	-2.57×10^{-4}	-3.31×10^{-4}	-1.82×10^{-4}
cg12961607	SRSF7	1.50×10^{-10}	-1.32×10^{-3}	-1.72×10^{-3}	-9.28×10^{-4}
cg15722438	HNRNPM	4.16×10^{-10}	9.55×10^{-4}	6.62×10^{-4}	1.25×10^{-3}
cg03309421	LSM5	5.99×10^{-10}	-3.50×10^{-4}	-4.58×10^{-4}	-2.42×10^{-4}
cg26543335	SFRS11	8.27×10^{-10}	-2.70×10^{-4}	-3.54×10^{-4}	-1.86×10^{-4}
cg15933130	HNRNPA0	1.89×10^{-9}	-2.74×10^{-4}	-3.61×10^{-4}	-1.86×10^{-4}
cg04441847	SRSF7	2.14×10^{-8}	-1.22×10^{-3}	-1.65×10^{-3}	-8.03×10^{-4}
cg25583580	SRSF2	2.16×10^{-8}	-5.83×10^{-4}	-7.84×10^{-4}	-3.83×10^{-4}
cg06201030	LSM2	9.03×10^{-8}	-4.22×10^{-4}	-5.75×10^{-4}	-2.70×10^{-4}
cg10601968	HNRNPA0	1.40×10^{-7}	-4.47×10^{-4}	-6.11×10^{-4}	-2.83×10^{-4}
cg07204893	SF3B1	1.84×10^{-7}	-6.98×10^{-4}	-9.56×10^{-4}	-4.39×10^{-4}
cg21197224	SRSF1	3.10×10^{-7}	-7.02×10^{-4}	-9.68×10^{-4}	-4.37×10^{-4}
cg20706192	HNRNPA2B1	7.75×10^{-7}	-2.49×10^{-4}	-3.47×10^{-4}	-1.52×10^{-4}
cg05412979	PNISR	1.12×10^{-6}	-3.00×10^{-4}	-4.19×10^{-4}	-1.81×10^{-4}
cg15123670	HNRNPH3	1.90×10^{-6}	-1.99×10^{-4}	-2.80×10^{-4}	-1.18×10^{-4}
cg18558763	SFRS14	1.92×10^{-6}	-1.90×10^{-4}	-2.67×10^{-4}	-1.13×10^{-4}
cg05491930	PNISR	3.16×10^{-6}	-4.85×10^{-4}	-6.87×10^{-4}	-2.83×10^{-4}
cg26744362	U2AF2	6.55×10^{-6}	-6.87×10^{-4}	-9.83×10^{-4}	-3.91×10^{-4}
cg17868445	HNRNPD	6.94×10^{-6}	-5.31×10^{-4}	-7.60×10^{-4}	-3.02×10^{-4}
cg06743844	SRSF7	1.90×10^{-5}	-1.83×10^{-4}	-2.65×10^{-4}	-9.96×10^{-5}
cg05012425	SRSF1	4.09×10^{-5}	-1.52×10^{-4}	-2.25×10^{-4}	-8.02×10^{-5}
cg19142553	HNRNPA2B1	4.70×10^{-5}	-5.77×10^{-4}	-8.53×10^{-4}	-3.02×10^{-4}
cg02583484	HNRNPA1	7.36×10^{-5}	-7.02×10^{-4}	-1.05×10^{-3}	-3.57×10^{-4}

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

ProbeID	Gene	P value	Coefficient	LCI	UCI
cg00899907	LSM2	7.87×10^{-05}	3.66×10^{-04}	1.86×10^{-04}	5.46×10^{-04}
cg25839482	IMP3	8.10×10^{-05}	-4.03×10^{-04}	-6.01×10^{-04}	-2.04×10^{-04}

Table 2
Correlations between DNA methylation and gene expression for a subset of splicing factor genes

Correlations between DNA methylation and expression levels for 27 age-associated splicing factors in 506 participants from the InCHIANTI are given. Gene and probe identities are indicated. Meth = methylation probe, Exp = expression probe. Coefficients, p values and 95% confidence intervals are given.

Gene	Meth Probe	Exp Probe	Coefficient	p value	Lower CI	Upper CI
SRSF3B1	cg07204893	ilmn_1706075	154.763	0.010	36.524	273.002
IMP3	cg25839482	ilmn_1733696	-219.188	0.021	-405.480	-32.897
U2AF2	cg26744362	ilmn_1768930	-59.442	0.063	-122.020	3.136
LSM2	cg00899907	ilmn_2070300	0.467	0.096	-0.083	1.016
SRSF18	cg05491930	ilmn_2161357	42.245	0.101	-8.262	92.752
HNRNPA1	cg02583484	ilmn_1661346	-0.298	0.118	-0.671	0.076
SFRS14	cg18558763	ilmn_1689007	-27.719	0.154	-65.889	10.451
SRSF7	cg06743844	ilmn_1778836	-29.994	0.264	-82.697	22.708
SRSF1	cg08591265	ilmn_1795341	-0.098	0.319	-0.292	0.095
SRSF1	cg05012425	ilmn_1795341	-0.392	0.356	-1.228	0.443
SFRS11	cg26543335	ilmn_1657790	21.061	0.377	-25.718	67.839
HNRNPA2B1	cg20706192	ilmn_1886493	0.345	0.394	-0.450	1.139
HNRNPA0	cg15933130	ilmn_1753279	-48.010	0.397	-159.227	63.206
SRSF7	cg04441847	ilmn_1778836	-5.425	0.411	-18.386	7.537
SRSF11	cg22718189	ilmn_1657790	11.889	0.433	-17.865	41.643
HNRNPH3	cg15123670	ilmn_1654920	-8.779	0.436	-30.904	13.347
HNRNPD	cg17868445	ilmn_2321451	-152.350	0.477	-572.920	268.220
SRSF1	cg21197224	ilmn_1795341	0.125	0.522	-0.258	0.508
SRSF18	cg05412979	ilmn_2161357	21.695	0.550	-49.499	92.889
HNRNPM	cg15722438	ilmn_1805371	-3.112	0.602	-14.834	8.610
LSM2	cg06201030	ilmn_2070300	-0.137	0.686	-0.802	0.528
HNRNPA0	cg10601968	ilmn_1753279	-11.530	0.718	-74.263	51.204
LSM5	cg03309421	ilmn_1737947	0.035	0.789	-0.222	0.291
SRSF1	cg23960707	ilmn_1795341	0.040	0.815	-0.296	0.377
SRSF2	cg25583580	ilmn_1696407	-0.090	0.818	-0.860	0.680
SRSF7	cg12961607	ilmn_1778836	0.262	0.969	-13.135	13.658

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Gene	Meth Probe	Exp Probe	Coefficient	p value	Lower CI	Upper CI
HNRNPA2B1	cg19142553	ilmm_1886493	-0.006	0.970	-0.296	0.285