Processed pseudogenes are non-functional copies of normal genes that arise by a process of mRNA retrotransposition. The human genome contains thousands of pseudogenes; however, knowledge regarding their biological role is limited. Previously, we demonstrated that high mobility group A1 (HMGA1) protein regulates the insulin receptor (INSR) gene and that two diabetic patients demonstrated a marked destabilization of HMGA1 mRNA. In this paper we report that this destabilization of HMGA1 mRNA is triggered by enhanced expression of RNA from an HMGA1 pseudogene, HMGA1-p. Targeted knockdown of HMGA1-p mRNA in patient cells results in a reciprocal increase in HMGA1 mRNA stability and expression levels with a parallel correction in cell-surface INSR expression and insulin binding. These data provide evidence for a regulatory role of an expressed pseudogene in humans and establishes a novel mechanistic linkage between pseudogene HMGA1-p expression and type 2 diabetes mellitus.
High mobility group A1 (HMGA1) is an architectural nuclear protein that binds to AT-rich regions of DNA and functions mainly as a specific cofactor for gene activation\(^1\). As part of an investigation into the molecular basis of regulation of the human INSR gene, we previously identified HMGA1 as a transcriptional regulator specifically needed for proper expression of this gene in eukaryotic mammalian cells\(^2\). Consistent with these findings, human type 2 diabetes (T2D) has been associated with defects in a nuclear regulatory protein either identical to, or highly related to, HMGA1, with commensurate decreases in INSR expression and consequent insulin resistance\(^3\).\(^4\).

In this study, we report on two unrelated patients affected by insulin resistance and T2D in whom the expression of HMGA1 was markedly reduced and INSR gene transcription was considerably impaired as assessed in Epstein–Barr Virus (EBV)-transformed lymphoblasts of both subjects. Patient 1, a non-obese 45-year-old Italian man with the usual features of T2D, came to medical attention because of fasting hyperglycemia and hyperinsulinemia, in the absence of other disorders such as acromegaly or glucocorticoid excess. Patient 2, an 11-year-old Japanese boy, was diagnosed with T2D at 6 years of age and had the male form of type A insulin resistance with acanthosis nigricans. By investigating the molecular events underlying the impaired expression of HMGA1 in both diabetic individuals, we have previously identified a heterozygous single-nucleotide G deletion in the 3′-untranslated region (UTR) of the HMGA1 gene, which seemed to be linked to the accelerated degradation of HMGA1 mRNA in patients’ cells\(^5\). However, when the mutant 3′-UTR was cloned into the 3′ position of a luciferase reporter gene and its expression was analysed \textit{in vitro} in transient transfection assays, luciferase activity decreased by only 50\%, suggesting that this mutation, although potentially contributing to the loss of HMGA1 mRNA, was not fully sufficient to account for the observed large decline in HMGA1 mRNA stability \textit{in vivo} in these subjects.

Here, we report the overexpression of a novel HMGA1 pseudogene in both diabetic individuals. This HMGA1-p RNA effectively competes with HMGA1 3′-UTR for a critical RNA stability factor. In this manner, the pseudogene RNA triggers a marked destabilization of HMGA1 mRNA with consequent loss of INSR expression and generation of the insulin resistance phenotype.

Results

Overexpression of the HMGA1-p pseudogene in diabetic patients. HMGA1 mRNA and protein abundances were determined in EBV-transformed lymphoblasts from diabetic patients and non-diabetic control subjects. In EBV-transformed lymphoblasts from both patients 1 and 2, HMGA1 mRNA and protein levels were markedly reduced compared with levels in non-diabetic controls (Fig. 1a). The decrease in HMGA1 expression in EBV-transformed lymphoblasts from patients 1 and 2 closely paralleled the decrease in INSR mRNA and protein levels. INSR mRNA and protein abundances were normal in EBV-transformed lymphoblasts from all T2D patients expressing normal levels of HMGA1 (Fig. 1a). The protein studies were carried out on nuclear extracts; HMGA1 protein was undetectable in cytoplasmic extracts (Fig. 1a). The protein studies were carried out on nuclear extracts; HMGA1 protein was undetectable in cytoplasmic extracts (Fig. 1a). The protein studies were carried out on nuclear extracts; HMGA1 protein was undetectable in cytoplasmic extracts (Fig. 1a).

Figure 1 | HMGA1, HMGA1-p and INSR expression levels. (a) Comparison of HMGA1 mRNA (grey bars) and protein content with INSR mRNA (white bars) and protein expression in cultured lymphoblasts from controls (C1, C2) and patients (P1, P2), C1, representative of 20 normal subjects; C2, representative of 20 T2D patients with normal INSR expression. Normalized mRNA is expressed as a percentage of maximal value (100\%). Results are the mean ± s.e.m. of three separate assays. *P<0.001 compared with controls. IP of the INSR and WB were performed using an anti-INSR β-subunit antibody. Sp1 and β-actin, control of nuclear and total cellular protein loading, respectively. (b) HMGA1 and HMGA1-p mRNA as detected by a unique set of primers in semiquantitative RT-PCR of cDNA from cultured lymphoblasts of patients (P1, P2) and non-diabetic (C1–3, representative of 20 healthy subjects) and diabetic (C4–5, representative of 20 T2D patients) controls. mRNAs from T2D patients with the same G deletion as P1 and P2, but without HMGA1-p overexpression (C6, representative of seven T2D patients) are shown. Values were standardized with ACTB mRNA. Genomic DNA contamination in RNA samples was excluded by PCR using aliquots of RNA without reverse transcription (−RT). Total RNA (5 μg) from the same set of cultured lymphoblasts was analysed by northern blot using cDNA probes for HMGA1 and HMGA1-p. Representative autoradiograms are shown.

HMGA1-p affects HMGA1 mRNA and INSR expression and function. The reciprocal relationship between HMGA1 and HMGA1-p
mRNA suggested that overexpression of the HMGA1-p pseudogene may result in decreased HMGA1 protein expression, thereby leading to the observed decrease in INSR expression and the diabetic phenotype in these individuals. To test this model, a recombinant plasmid carrying the entire HMGA1-p pseudogene was generated and transiently transfected into cultured human HeLa cells, a cell line naturally expressing the homologous HMGA1 coding gene. As shown in Fig. 2a, treatment of HeLa cells with increasing amounts of HMGA1-p-containing plasmid caused a dose-dependent decrease in HMGA1 mRNA levels, with the maximal inhibition (50–60%) occurring at 2 μg recombinant vector. This reduction was specific for HMGA1, as mRNA expression for the ubiquitously expressed transcription factor Sp1 was unaffected. Reduced HMGA1 mRNA levels paralleled the decrease in HMGA1 protein levels as detected by western blot (WB) analysis of nuclear extracts from transfected HeLa cells (Fig. 2a). Similar results were obtained in human HEK-293 cells, which do not express appreciable levels of endogenous HMGA1. As shown in Fig. 2a, HMGA1 mRNA and protein levels were impaired in HEK-293 cells when both HMGA1 and HMGA1-p were coexpressed simultaneously. When forced expression of HMGA1-p was induced in 3T3-L1 adipocytes (and in other insulin-sensitive human and mouse cell lines, such as HepG2 and Hepa-1 liver cell lines), HMGA1 and INSR expression levels were reduced, and this reduction was associated with a decrease in insulin signalling events, such as insulin-stimulated INSR β-subunit auto-phosphorylation and the phosphorylation of the insulin receptor substrate 1 (IRS1) (Fig. 2b).

Accumulating evidence strongly implicates posttranscriptional mechanisms involving mRNA structure (for example, folding) and mRNA stability in the regulation of gene expression. To determine whether HMGA1-p overexpression in patients 1 and 2 was involved in posttranscriptional processes affecting HMGA1 expression, we examined the half-life of HMGA1 mRNA in the EBV-transformed lymphoblasts of patients. HMGA1 mRNA half-life decreased from ~26 h in control cells to 4–6 h in cells of diabetic individuals overexpressing HMGA1-p (Fig. 2c). When studies of mRNA decay were conducted in patients’ cells transfected with small interfering RNA (siRNA) specific to HMGA1-p, HMGA1 mRNA was stabilized, exhibiting a half-life of 10–12 h (Fig. 2c), along with a parallel increase in HMGA1 mRNA abundance (Fig. 2d). These data

![Figure 2](image.png)

**Figure 2** | Impact of HMGA1-p on HMGA1 mRNA levels and stability and on INSR expression and function. (a) HeLa and HEK-293 cells were transfected as indicated, and HMGA1 (grey bars) and control Sp1 (white bars) mRNA and protein levels were measured by real-time PCR and WB, respectively. mRNA is expressed as a percentage of max (100%, untransfected HeLa cells or HEK-293 cells plus HMGA1 alone). Results are the mean ± s.e.m. of three experiments. *P < 0.05 versus untransfected HeLa cells; **P < 0.05 versus HEK-293 cells overexpressing HMGA1 alone. (b) Left: HMGA1 (grey bars), INSR (white bars), Sp1 (black bars) and β-actin (slashed bars) mRNA and protein (IP/WB), as measured in 3T3-L1 adipocytes, before and after transfecting HMGA1-p expression vector. Right: INSR and IRS1 expression and INSR β-subunit (pINSRβ) and IRS1 (pIRS1) phosphorylation were determined by IP/WB of insulin-stimulated lysates from untransfected and transfected cells. White bars, pINSRβ; black bars, pIR1. Densitometric results are the mean ± s.e.m. Results are from three independent experiments. *P < 0.05 versus untransfected 3T3-L1 cells. (c) HMGA1 mRNA decay in patients and control cells treated with non-targeting siRNA (solid lines), or HMGA1-p siRNA (dashed lines). Open square, control (representative of 20 normal subjects); solid diamond, patient 1 (P1); solid circle, patient 2 (P2). mRNA amount at each time point is expressed as a percentage of max (100%, time 0), plotted on semilog axes. Results are the mean ± s.e.m. of triplicates from three independent experiments. (d) HMGA1-p (black bars), HMGA1 (grey bars) and INSR (white bars) mRNA in cultured lymphoblasts from controls (n = 20) and patients 1 and 2, after treatment with control/HMGA1-p siRNA. Results are means ± s.e.m. of three real-time PCRs. *P < 0.05 versus untreated cells, per condition. HMGA1 and INSR protein levels are shown in representative IP/WB of three independent experiments. (e) Insulin binding to EBV-transformed lymphoblasts pretreated with control/HMGA1-p siRNA. Binding is expressed as percentage of total/10^6 cells. Results are the mean ± s.e.m. of three separate experiments. C1, healthy subjects (n = 20); C2, T2D patients with normal HMGA1 expression (n = 20); P1 and P2, patients 1 and 2. *P < 0.001 versus control (C1, C2) cells; **P < 0.05 versus untreated patient’s (P1, P2) cells.
revealed an inverse correlation between HMGA1-p pseudogene expression and expression of its homologous HMGA1 coding gene in these individuals. Interestingly, the partial restoration of HMGA1 mRNA levels after treating cells with siRNA against HMGA1-p was accompanied by a concomitant increase in INSR mRNA and protein expression levels (Fig. 2d), and this increase was followed by a restoration of cell-surface insulin binding in patients’ cells (Fig. 2e). The fact that transfection of patients’ cells with siRNA to HMGA1-p significantly, but not completely, corrected HMGA1 mRNA stability and levels is consistent with the possibility that G deletion in the 3′-UTR of the HMGA1 gene, in these patients, can synergize with HMGA1-p overexpression in inducing HMGA1 mRNA destabilization/degradation.

**Impact of HMGA1-p 3′-UTR on HMGA1 mRNA expression.** To further explore the role of HMGA1-p in the control of HMGA1 mRNA stability, we mapped the segment of the HMGA1-p RNA that is critical to this pathway. Cells were transiently transfected with recombinant vectors carrying either the full-length or the truncated version of HMGA1-p. As shown in Figure 3a, transfection of HeLa cells with the recombinant plasmid HMGA1-p lacking the 248-bp 5′ region (corresponding to the 5′-UTR of HMGA1 gene) caused a 50% decrease in HMGA1 mRNA expression. This reduction was similar to the reduction obtained by transfecting full-length HMGA1-p. In contrast, no decrease in HMGA1 expression was observed in cells transfected with plasmid containing HMGA1-p lacking the 1,276 bp 3′ region (corresponding to the 3′-UTR of the HMGA1 gene) (Fig. 3a), suggesting that cis-acting element(s) in the 3′ region of HMGA1-p (is) are responsible for the observed impact on HMGA1 mRNA instability/degradation. This observation is consistent with our previous results indicating that HMGA1 3′-UTR has critical posttranscriptional regulatory elements within its 3′-UTR. To characterize in greater detail those regions of the HMGA1-p 3′-UTR that were implicated in posttranscriptional processes affecting HMGA1 mRNA stability/degradation, several deletion mutants of HMGA1-p 3′-UTR, containing homologous regions of HMGA1, were cloned into the pcDNA 3 plasmid vector, and analysed in transient transfection assays for their ability to interfere with transcription of the homologous HMGA1 gene. Real-time RT-PCR analysis revealed that the expression level of endogenous HMGA1 was only slightly increased in HeLa cells, in the presence of recombinant plasmids bearing proximal deletion mutants of HMGA1-p 3′-UTR (Δ291–Δ1026) (Fig. 3b). In contrast, HMGA1 mRNA abundance was considerably decreased in the presence of recombinant constructs containing the distal deletion mutants of HMGA1-p 3′-UTR (Δ1253 and Δ1276) (Fig. 3b). As shown in Fig. 3b, deletion of 152 bp of the 3′ end of the HMGA1-p 3′-UTR (region 4) reduced HMGA1 expression to approximately 40–50%, suggesting the presence, within this region, of elements that can decrease endogenous HMGA1 mRNA expression in trans. A possible mechanism for this control may be titration by HMGA1-p RNA of one or more trans-acting cytoplasmic protein factors critical to HMGA1 mRNA stability.

**Physical association of αCP1 with the HMGA1 and HMGA1-p 3′-UTR.** To better understand the mechanism underlying the ability of HMGA1-p to reduce HMGA1 mRNA stability and induce HMGA1 mRNA degradation, we carried out a series of RNA-electrophoretic mobility shift assay (EMSA) combined with pull-down and immunoblot assays to identify the corresponding shared RNA-binding proteins. As shown in Figure 4a, RNA–protein binding activity was detected with both HMGA1 (H7) and HMGA1-p (H7-p) biotinylated 3′-UTR mRNA probes, in the presence of increasing amounts of cytoplasmic extracts from HeLa cells. To determine the specificity of RNA–protein binding, competition assays were performed. A 30-fold excess of either unlabelled H7 or unlabelled H7-p significantly reduced the binding of labelled H7 and labelled H7-p to the RNA-binding proteins, respectively, whereas the presence of the same amount of nonspecific competitor RNA had no significant effect on the complex formation (Fig. 4a). Moreover, unlabelled H7-p competitively inhibited the binding of labelled H7 to cytoplasmic proteins (Fig. 4a). In concert with this observation, unlabelled H7 inhibited the binding of labelled H7-p to cytoplasmic proteins (Fig. 4a). As this region of 3′-UTR mRNA contains three proximal and potentially important C-rich stretches, additional competition experiments were carried out with the synthetic homoribopolymer poly(C). Interaction of HeLa cytoplasmic proteins with either H7 or H7-p was markedly decreased in the presence of increasing amounts of poly(C) (Fig. 4b). This effect was specific, as no appreciable interference was detected in the presence of homoribopolymers poly(A), poly(G) or poly(U), and this specificity was further confirmed by lack of RNA–protein binding on incubation with the H7 probe in which mutations within the C-rich stretches were generated (Fig. 4b). On the basis of these observations, we hypothesized a role in RNA–protein binding activity for αCP1 proteins, a group of KH-domain RNA-binding proteins with binding specificity to C-rich patches, which have prominent roles in mRNA stability control11. Consistent with this hypothesis, the unique protein–RNA complex in HeLa cells was recognized and supershifted to a slower-migrating form by incubation with an affinity purified antisera to the C-binding protein, αCP1 (FF1) (Fig. 4c). These data were substantiated by pull-down and RNA immunoprecipitation (IP) of αCP1–RNA complexes from HeLa cell extracts (Fig. 4d), suggesting that αCP1 binds in vivo, as well as in vitro, to HMGA1 and HMGA1-p RNAs. In agreement with these observations, HMGA1 mRNA and protein levels were decreased in HeLa cells following siRNA-mediated knockdown of endogenous αCP1 expression (Fig. 4e).

**Discussion**

Processed pseudogenes result from reverse transcribed mRNAs12. In general, because pseudogenes lack promoters, they are no longer
functional from the moment they are inserted into the genome and are not transcribed into mRNA. However, recent evidence indicates that a subset of processed pseudogenes may perform regulatory functions. The ENCODE project has estimated that ~20% of pseudogenes are transcribed with a pattern of tissue or cell line specificity, and a number of functional pseudogenes have been shown to be indispensable for the evolution of mammals and can affect gene expression. For example, it has been shown that, in Lymnea stagnalis, a pseudogene homologous to the nitric oxide synthase gene decreases the expression levels of its normal gene of origin through the formation of an RNA duplex that arises through a reverse-complement sequence found at the 5’ end of the pseudogene transcript. In a second example, transcription of the makorin1-p1 pseudogene in mouse was required for the stability of mRNA from a homologous gene makorin. Notwithstanding, this latter study has been subsequently challenged, and a biological function for transcribed pseudogenes has been confirmed in several successive studies. Despite multiple lines of evidence supporting functional role(s) of pseudogenes in mammals, evidence of a role of such interactions in human disease has heretofore been lacking.

In this study, we describe a novel human HMGA1 pseudogene, HMGA1-p, which regulates mRNA stability of its corresponding coding gene. The data are consistent with a model by which the HMGA1-p pseudogene transcript effectively competes for a trans-acting cytoplasmic protein critical to the longevity of HMGA1 mRNA. By accelerating the degradation of HMGA1 mRNA transcript, the elevated levels of HMGA1-p RNA in affected individuals suppress expression of the INSR gene, resulting in insulin resistance and T2D. Previous studies have demonstrated that HMGA1 gene expression is posttranscriptionally regulated through signals...
EBV-transformed lymphoblasts were established from patients and from non-diabetic (n = 20) and diabetic (n = 27) individuals (Istituto G. Gaslini, Genova, Italy, Telethon Genetic Biobank Network, Project GTB07001A). All lymphoblastoid cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and were grown in similar conditions in all labs. HeLa (HeLa and HEK-293) and mouse (3T3-L1) cell lines (ATCC) were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. 3T3-L1 fibroblasts were differentiated into 3T3-L1 adipocytes according to a standard protocol. In studies of insulin signalling, 3T3-L1 cells were deprived for 3h and treated with insulin for 10 min before preparation of whole-cell lysates. IP and WB of INSRR and HMGA1 and Sp1 were performed on total cellular extracts and nuclear extracts from EBV lymphoblasts and human and mouse cultured cells. Total and tyrosine-phosphorylated INSRR and IRS1 were assayed in 3T3-L1 cells after IP of cell lysates using INSRR and IRS1 antibodies, followed by immunoblotting using an antiphosphotyrosine antibody. The antibodies were used against HMGA1α, anti-INSRR, anti-Sp1, anti-β-actin (Santa Cruz Biotechnology); anti-phosphotyrosine and anti-IRS1 (Upstate Biotechnology).

**RT-PCR, northern blot, and mRNA decay.** Total cellular RNA was extracted from cells using an RNAsafe-PCR kit and subjected to DNase treatment. RNA levels were normalized against 18S ribosomal RNA in each sample. Complementary DNA was synthesized from total RNA with a RETROscript first strand synthesis kit (Ambion) and used for PCR amplification. Northern blots and hybridizations were carried out following standard procedures. Probes for HMGA1 and HMGA1-p were obtained by amplifying the respective 3′-UTR non-homologous regions. HMGA1-p signals were quantified with Molecular Dynamics PhosphorImager and ImageQuant software (Molecular Dynamics). For the half-life of HMGA1 mRNA, logarithmic-phase EBV lymphoblasts were exposed to 0.5 μg ml⁻¹ of actinomycin D. RNA was extracted at 4-h intervals, cDNA was prepared and HMGA1 mRNA levels were measured by quantitative RT-PCR, using RPS9 mRNA as control. mRNA half-life was determined by regression analysis and visual determination of the point at which the best-fit line crossed the 50% intercept.

**Transfections and 32P-labelled insulin binding.** pG3 control vector, containing HMGA1 or HMGA1-p, was transfected into either HeLa, HEK-293 or 3T3-L1 cells, using LipofectAMINE 2000 reagent (Invitrogen), and HMGA1 and INSRR mRNA and protein expression levels were assayed 48–96h later. For siRNA experiments, 100–200 pmol of siRNA targeted to HMGA1-p (Invitrogen) was transfected into EBV-transformed lymphoblasts (4×10⁵ cells) using the diethylaminoethyl (DEAE) dextran method, and nuclear extracts or total cellular lysates were prepared 72 h later. RNA (100–200 pmol) was used to knock down CP1 in transfected HeLa cells. A nonspecific siRNA with a similar GC content (Dharmacon) and a nontargeting scrambled sequence (Santa Cruz Biotechnology) were used as a control. 32P-labelled insulin binding to EBV-transformed lymphoblasts treated with siRNA to HMGA1-p was measured 72 h after transfection.

**EMSA and biotin pull-down assays.** The 3′ end of both HMGA1 (H7) and HMGA1-p (H7-p) cDNA containing the appropriate T7 promoter sequence was amplified by PCR and 1 μg of PCR product was transcribed using the Ambion T7-Flash Transcription Kit (Epitect) with a portion of the UTP replaced by 125I-UPT probe incubated with Biotin-16-UTP (Ambion). Biotin-labelled RNA probes (500 fmoles each) were incubated with HeLa cytoplasmatic extracts and RNA–protein binding and pull-down assays were performed. Briefly, the RNA probe was incubated for 1 h with protein extract (5 μg) in 1 x binding buffer (10 mM Hepes, 40 mM KCl, 3.0 mM MgCl₂, 1 μM dithiothreitol, 5% glycerol, 1 μg tRNA and 1.25 μg ml⁻¹ heparin) at room temperature. The binding reaction was subsequently incubated with RNase A (10 ng μl⁻¹, Qiagen) for 10 min and 1 μg heparin (50 μg ml⁻¹) was added to each reaction mixture. RNA–protein complexes were separated 10 min later on SDS-PAGE (6%) and transferred to Hybond-N+ membranes. Signal of RNA–protein binding was detected using the LightShift chemiluminescent kit (Pierce). FF1 antisense antibody (against CP1) was used in supershift experiments. RNA used as nonspecific competitor was an oligonucleotide of unrelated sequence (Supplementary Table S1) obtained by PCR using the AmpliScript T7-Flash Transcription Kit, in the presence of T7 promoter sequence. RNA pull-down assays were carried out with 2 μg of either H7 or H7-p biotinylated probe coupled to Streptavidin-coated M280 Dynabeads (Invitrogen). HeLa cell lysates (100 μg) plus tRNA (30 μg) were added to RNA beads and incubated for 30 min at 4°C. At the end of the incubation period, the supernatant was removed, the beads were washed three times with 100 μl of hypotonic buffer, resuspended in 20μl 1 x Laemmli lysis buffer and analysed by WB analysis using FF1 antisense.

**RNA IP assay.** HMGA1 mRNA was detected by RT–PCR, after RNA IP, using anti-cp1 antibody FF1. HeLa cells (3x10⁵) were lysed in IP buffer (50 mM Tris-HCl, pH 7.4/1% Nonidet P-40/0.5% sodium deoxycholate/0.05% SDS/1 mM EDTA/150 mM NaCl) containing protease inhibitors (Sigma) and 50 μl⁻¹ Superasin RNase inhibitor (Ambion), centrifuged at 12,000 g for 10 min at 4°C, after which the supernatant (0.75 ml) was added to 10 μg of FF1 antisense antibody. After 2 h of incubation at room temperature, an equal volume of protein A/G agarose beads was added and the mixture was incubated for an additional
The protein A/G agarose–antibody–protein complexes were washed with IP buffer, bound RNAs were extracted using the RNAqueous–4PCR Kit and subjected to DNase treatment (Ambion); thereafter, HMGAI mRNA was detected by RT–PCR.

The studies on humans were approved by the local ethics committee (Comitato Etico Regione Calabria, Azienda Ospedaliera ‘Mater Domini’, Catanzaro). We obtained informed consent from all individuals or from their parents. Statistical analysis was performed using the Student’s t-test. For all analyses, P < 0.05 was considered significant.

DNA and RNA sequences are described in Supplementary Table S1.

References


Acknowledgments

We are most grateful to F.S. Brunetti for his help in artwork preparation. We also thank A. Malta, T. Rossano, G. Cervalvo and G. Grandinetti for secretarial help. This work was supported by Telethon-Italy, Grant GGP04245, and MIUR, protocol 2004062059-002 Italy, to A. Brunetti.

Author contributions

E.C. conceived the study and contributed to the writing of the paper. S.I. and L.L.P performed transfection assays; E.C. and F.P. conducted studies with EBV-transformed lymphoblastoid cell lines from human blood samples; D.F. conducted studies on mRNA decay; S.A.L. contributed to the data analysis and revision of the paper. A.B. coordinated and supervised the study, analysed the data and wrote the paper. All authors discussed the results and commented on the paper.

Additional information

Accession numbers: HMGAI mRNA variant 5: NM_145903; HMGAI-p: AC005041; HMGAI mRNA variant 1: NM_145899; HMGAI: NT_007592.15.

Supplementary information accompanies this paper on http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Chieffari, E. et al. Pseudogene-mediated posttranscriptional silencing of HMGAI can result in insulin resistance and type 2 diabetes. Nat. Commun. 1:40 doi: 10.1038/ncomms1040 (2010).