Table 2
Examples of exploratory questions and empathic statements.

Exploratory questions	Empathic statements
How do you mean?	*I can see how upsetting this is to you.*
Tell me more about it.	*I can tell you weren't expecting to hear this.*
Could you explain what you mean?	*I know this is not good news for you.*
You said it frightened you?	*I'm sorry to have to tell you this.*
Could you tell me what you're worried about?	*This is very difficult for me also.*
Now, you said you were concerned about your children. Tell me more.	*I was also hoping for a better result.*

could seem obvious what the patient feels but translating it into words is a way to rationalize it together.

3. Identify the reason for the emotion (exploratory questions in Table 2). Once again you will wonder why you should do it, because it is obvious that patient is feeling like that due to the bad news about his health. Maybe it is, but he has to say it out loud, and maybe there could be many other reasons, fears and expectations that lie behind. The disease could be the cause of several different problems that are significant for the patient, such as loss of a job or inability to take care of family.
4. Give the patient a brief period of time to express his feelings and then let the patient know that you understood why he feels so by making a statement that reflects his understanding. If you want, you can also make statements to acknowledge your own emotions (empathic statements in Table 2).

In addition, you can show your support also by making validating statements, to let the patient know that these feelings are legitimate. This last is one of the most effective approaches in communication: feeling gotten and legitimated will help the patient to open up and trust you. E.g.:

√ *I guess anyone might have the same reaction.*
√ *You were perfectly correct to think that way.*

STEP 6: strategy and summary

When you have to face a problem, knowing which is your plan will help you manage stress. The same is true for a patient who has to cope with a disease. Building a strategy is important for a number of reasons: it helps patients to manage stress and fear, it boosts confidence into patient physician relationship, but most of all it is a staple of the establishment of patient's autonomy, that goes far beyond the legal mandate of informed consent. Below find some practical tips:

√ Before discussing a treatment plan ask the patient if he is ready for such a discussion.

√ Let the patient feel you are sharing responsibility for decision making.

√ Check the patient's misunderstanding of the discussion, to prevent the documented tendency of patients to overestimate the efficacy or misread the purpose of the therapy.

√ Understand what specific goals are important to the patient (such as symptom control).

√ Remember to frame hope in terms of what it is possible to accomplish.

√ Do not be scared to let the patient understand that every medical intervention, even the safest one, has a probability to be unsuccessful: if patients are to truly share in decision making, they have to know. Physicians often fear that by expressing uncertainty, they will project ignorance to patients, so they internalize and mask it [11]. At the same time patients, scared of the unknown, quest for certainty. Yet the reality is that physicians continually have to make decisions on the basis of imperfect data and limited knowledge, which leads to diagnostic uncertainty, coupled with the uncertainty that arises from unpredictable patient responses to treatment that are far from binary.

Finally, one last key advice: following a course of action that you learned will be comfortable and reassuring, but remember that, regardless the approach or the model you will choose, you need to practice and interiorize it so much that you will be able to use it automatically when you will be tired, nervous or angry, because knowing in theory what you have to do is much different than being able to do it under stress.

Conflict of interest

None declared.

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A severe hemojuvelin mutation leading to late onset of *HFE2*-hemochromatosis

Dear Editors,

HJV is a Bone Morphogenetic Protein co-receptor mainly expressed in the liver [1] that is required to drive *HAMP* transcription via SMAD proteins [2]. In humans, *HAMP* and *HFE2* mutations lead to juvenile hemochromatosis (JH) that usually manifests with hypogonadism and cardiomyopathy in the second-third decade of life leading, if untreated, to death because of heart failure at about 30–40 years [3]. Differently from the classical and more common HFE-related hemochromatosis, males and females are equally affected [3]. We report the case of a woman with a late-onset form of type 2a hemochromatosis due to a novel mutation in *HFE2* (p.Cys317Ser).

The 68 years-old woman was hospitalized because of acute heart failure. Since the age of 35 she complained for arthralgias at II and III metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints, subsequently involving metatarsal-phalangeal joints, knees and hips, occasionally treated with non-steroideal anti-inflammatory drugs (NSAID). At 49 years a diagnosis of serological negative rheumatoid arthritis was done, and hydroxyclorochin was added to NSAID. At 61 years she had a left hip replacement. At that time hyperferritinemia was noted, but no diagnostic work-up was performed. One year after, X ray scan showed alterations of the II and III MCP (cystic lesions and hook-shaped osteophytes). At 63 years she was diagnosed with diabetes (glycemia 172 mg/dL; glycosylated hemoglobin 6.7%) and treated with insulin because of low C-peptide level. Because of palpitations, a full cardiologic evaluation was done showing normal cardiac function. She began a symptomatic therapy with propranolol 20 mg twice a day. Serum ferritin levels and transferrin saturation were 4121 μg/L and 91%, respectively. *HFE* genotyping was negative for p.C282Y mutations. A liver biopsy was proposed, but the patient denied it. At the age of 68 years, she complained of worsening dyspnea due to acute cardiac failure requiring hospitalization. She was treated with intravenous diuretics, vasodilators and positive airway pressure ventilation with benefit. She was a lean woman (weight 48 kg and height 152 cm) with brown-grey pigmentation. She did not smoke and drunk less than 10 g of alcohol per day. She had four brothers and one daughter aged 37 in good health. She went to menopause at 49 years. Laboratory test showed mild anemia (Hb: 11.4 g/dL), hyperglycemia (179 mg/dL), normal renal and liver function tests, marked increase of serum ferritin (3987 μg/L) and transferrin saturation (99%). During hospitalization she developed recurrent atrial fibrillation events, treated with digitalis, beta-blockers and eventually, with amiodarone. Diagnostic work-up showed dilated cardiomyopathy with ejection fraction of 35%, confirmed insulin-dependent diabetes and arthropathy of MCP, PIP, knees and right hip. After clinical stabilization, a liver biopsy was done showing massive iron overload involving hepatocytes, Kuppfer cells, portal macrophages, endothelial and biliary cells (Total Iron Score: 45; Hepatic Iron Score: 27; Synusoidal Iron Score: 6; Portal iron Score 10), and cirrhosis. A clinical diagnosis of non-HFE hemochromatosis was done. Due to heart failure, she was treated with monthly erythrocytoaferesis with EPO injections (150 U/Kg/week) according to previous reported schedule [4], associated with subcutaneous deferoxamine 1 g five times a week. Because of the occurrence of a hepatic nodule at ultrasound after 6 months, EPO was discontinued despite ultrasound-guided biopsy excluded hepatocellular carcinoma. She continued with deferoxamine 1 g/day for 5 times a week for one year that was progressively reduced according to the decrease of serum ferritin level. After 5 years and removal of about 11 g of iron (IR), serum ferritin was 79 μg/L and transferrin saturation 83%. Heart function normalised and after 12 years of follow-up she is still alive. Extensive search for mutations in non-HFE hemochromatosis genes and functional studies were performed.

Her genomic DNA was isolated from peripheral blood leukocytes and after libraries preparation with HaloPlex Target Enrichment kit (Agilent Technologies, Santa Clara, CA, USA) it was sequenced on Ion Torrent PGM (Thermo Fisher Scientific, Waltham, MA, USA) using a custom panel of 26 genes currently associated with iron homeostasis and genetic iron overload or anemia. Sanger sequencing was used to confirm NGS findings. The proband and her relatives gave written informed consent to genetic testing analysis according to the Ethical Committee of our Institution. Different available online tools (DNA to protein sequence converter, ClustalW2, PolyPhen, ExPASy, SIFT and MUTPRED) were used to analyse mutations at genomic and protein levels. A sample of the liver biopsy, performed for diagnostic purposes, was used for RNA extraction and cDNA synthesis. mRNA expression of hepatic genes was evaluated by quantitative real time PCR using *HPRT1* as housekeeping gene. Urinary and serum hepcidin measurements were performed by SELDI-TOF mass spectrometry as previously described [5,6]. Mutant-h*HFE2* (p.Cys317Ser) plasmids were generated from pcDNA3.1-HJV^{MYC} and pcDNA3.1-HJV [7,8]. HeLa and Hep3B cells were transiently transfected with wild-type or mutant-h*HFE2* constructs and then harvested for (a) protein isolation and western blot analysis [8]; (b) analysis of cell surface expression of hemojuvelin by using binding assays [8]; (c) analysis of cell surface expression of hemojuvelin by using biotinylation assay; (d) Hepcidin promoter luciferase assay [7]. Major details about methods will be available on request.

We found a novel missense mutation in *HFE2* (c.1188G > C) at the homozygous state (Fig. 1A). The mutation changes a cysteine to serine at position 317 (p.Cys317Ser) in the ectodomain of HJV, close to the furin consensus cleavage site. Multiple alignment analysis (Fig. 1B) confirmed that the cysteine is highly conserved across species and *in-silico* software predicted this variant as "probably damaging". No mutations were found in the other iron-related genes such as *HFE*, *HAMP*, *SLC40A1* and *TFR2*. Proband's daughter and one of the four brothers were heterozygous for the mutation, while the others carried a wild-type genotype.

To analyse the biogenesis of WT and mutant HJV, we insert the mutation in the pcDNA3.1-HJV^{MYC} and pcDNA3.1-HJV expressing vectors [7,8]. The C317S variant and the wild-type protein (HJV^{wt}) were equally expressed, suggesting that the aminoacidic change does not interfere with the correct translation of the protein (Fig. 2A, upper panel). The HJV^{wt} isoform is normally cleaved at the GDPH consensus sequence in the von Willebrandt factor type D domain at position 172. Accordingly, it shows a smaller band of approximately 33 kDa, deriving from the GDPH cleavage, and the bigger 50-kDa species; at difference with the HJV^{wt} isoform, C317S variant lacks the 33 kDa form, suggesting an abnormal proteolytic processing (Fig. 2A, upper panel) [7,8]. Moreover, the C317S variant does not release soluble HJV in the culture media (Fig. 2A, lower panel). Also, we showed a strong reduction of biotinylated membrane HJV^{C317S} (Fig. 2B) and a decreased amount of membrane HJV^{C317S} assessed by the specific binding assay (Fig. 2C) confirming that the substitution heavily affects the correct processing of HJV [7,8]. Consistent with the role of HJV as BMP coreceptor, the C317S mutant was unable to activate hepcidin expression in a luciferase reporter vector (Fig. 2D), and the affected patient showed markedly reduced hepatic *HAMP* mRNA level by qRT-PCR ($2^{-\Delta\Delta Ct}$: 0.36 UA), and serum and urinary hepcidin [s-hepcidin: 1.53 ng/mL and U-hepcidin 4 ng/mg creatinine; normal mean values (range): 10.8 ng/mL (7.5–15) [6] and 44 ng/mg creatinine (10–200) [5], respectively].

Type 2a hemochromatosis is generally considered a fully penetrant disease [9]. At our knowledge there is only one paper describing a middle-age (around 50 years) onset of hemochromatosis due to mutations of *HFE2* in three Japanese patients [10], but this is the first report of an old-age onset of the disease. Similarly to Koyama et al. [10] we excluded that our proband had the early pituitary hypogonadism that typically characterises JH presentation [3], as she had a baby at the age of 31 and reached the menopause at 49 years. She first presented at the age of 68 with acute heart failure showing a hemochromatosis phenotype resembling a severe adult form, characterized by liver cirrhosis, insulin-dependent diabetes and cardiomyopathy. There were no acquired causes able to counteract the expression of the disease: the patient was lean, was not vegetarian or vegan or blood donor, had no history of heavy menstrual bleeding or chronic blood losses, and had a single pregnancy. Because of the previous (and probably mistaken) diagnosis of reumathoid arthritis, we accurately revised her previous and present medical charts and we excluded she ever had activation of

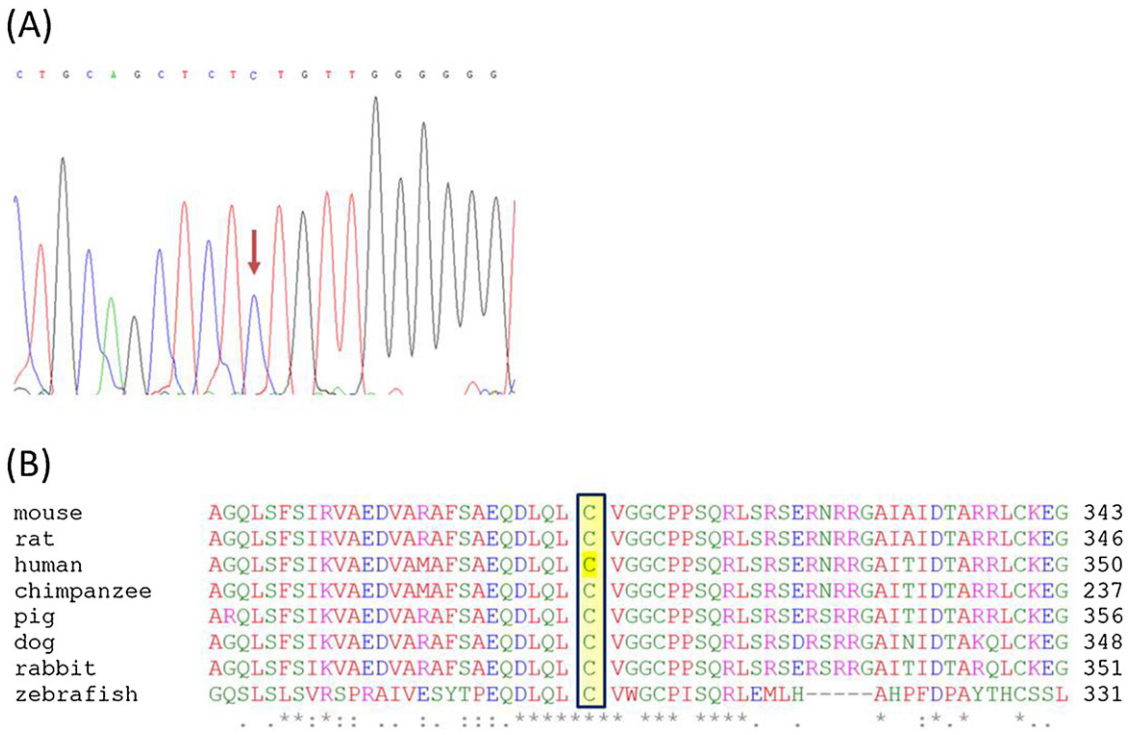

Fig. 1. (a) Electropherogram; (b) Fragment of multiple alignment around position 317 of HJV in different species.

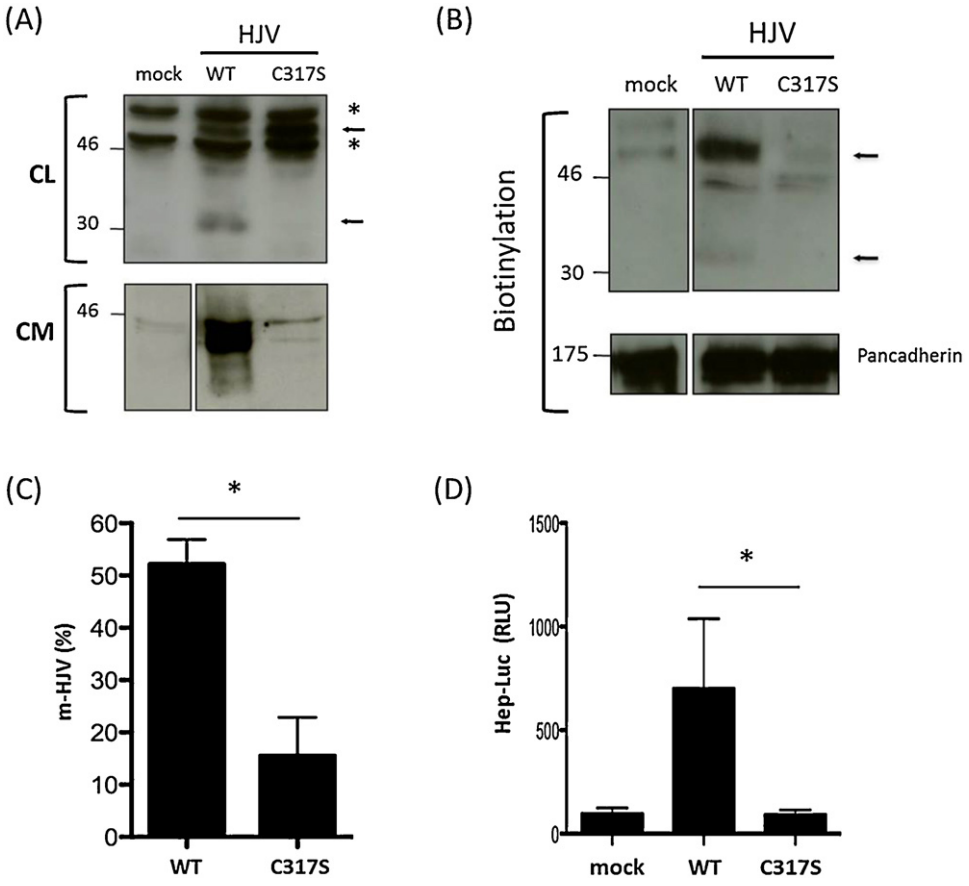

Fig. 2. (a) Western Blot of cell lysate (CL) and conditioned media (CM) normalised to unspecific band (*), arrows indicate the two HJV isoforms; (b) cell surface biotinylation, arrows indicate HJV; (c) binding assay; (d) hepcidin luciferase reporter assay.

inflammatory indices (erythrocyte sedimentation rate, C-reactive protein and alpha 1 and 2 globulins). To evaluate whether this uncommon presentation was related to a mild mutation, we performed an extensive functional study of the mutated protein that excluded the hypothesis. In fact, in cultured cells the mutated protein was unable to undergo the auto-proteolytic process and reach the cell membrane, release soluble HJV in the culture media, and activate hepcidin in a luciferase reporter vector. All these functions are essential for protein activity. Overall, these findings suggest the existence of other factors (gene modifiers) partially blunting the effect of the *HFE2* mutation, and that *HFE2* mutations can sometimes lead to non-juvenile form of hemochromatosis suggesting that *HFE2* genetic testing should not be restricted to young patients with severe iron overload.

Conflict of interest

None declared.

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