Investigation for a heterozygous effect was done on the variants Arg142His, Arg151Cys, Arg160Trp, Asp294His (which were chosen because they had been associated with red hair in previous case-control studies, and because they show a diminished ability to activate cyclic AMP in transfection assays,[4] and on an insertion at codon 179 (ins179), which results in a predicted premature stop codon and is likely to be non-functional. Other (pseudo wild-type) variants were treated as wild type. Detection of a heterozygote effect was sought by fitting a cumulative proportional odds model by Stata. This process automatically takes into account the ordering in the response. The effect of genotype was assessed by inclusion of genotype as a factor in the analysis, firstly as a linear trend from wild type through heterozygous to homozygous, and secondly as the remaining non-linear trend.

Individuals with one variant allele were intermediate with regard to skin type and the ability to tan after repeated sun exposure between those with two variant alleles and those with none of the variants (figure). Analysis for trend from zero to two variants was highly significant ($p<0·001$), for both depth of tanning and skin type, with little evidence of any non-linear trend ($p=0·844$ and $p=0·624$ for depth of tanning and skin type, respectively). The odds of having skin type below any given category were 3·82 times greater in heterozygotes than in individuals with wild-type alleles (95% CI 2·22–6·59), and 14·6 times greater in homozygotes or compound heterozygotes than in wild-type individuals (4·9–43·4). For tanning, the respective odds ratios were 4·5 for heterozygotes (2·54–7·99) and 20·3 for homozygotes or compound heterozygotes (6·4–63·9).

The identification of a dosage effect of *MC1R* variant alleles on sensitivity to ultraviolet radiation, and the large attributable risk for heterozygotes (28% of the study population were heterozygous for Arg142His, Arg151Cys, Arg160Trp, or Asp294His variant alleles) suggests that the *MC1R* gene is closely associated with variation in the skin's response to ultraviolet radiation in most of the population who do not have red hair. Furthermore, because *MC1R* heterozygotes are common, the results suggest that the *MC1R* gene is of substantial importance as a susceptibility gene for sunburn, photoageing, and skin cancer.

Pigmentation is a complex genetic trait, and our results have implications for studies on other complex genetic disease states. Because a large number of loci can affect pigmentation in mice, and a large number of rare Mendelian disorders affect pigmentation in man, a large number of loci may mistakenly be assumed to underlie "physiological" differences in pigmentation within human populations; in this scenario each locus would contribute, on average, a small fraction of the overall variance. However, for *MC1R*, the effect of one locus in man is substantial, and our results show that large risk ratios can be detected for common alleles where an adequate phenotypic classification of the disease or trait is available. Such variation is likely to outweigh the effect of rare Mendelian disorders on population attributable risk.

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1 Gallagher RP, Ho VC. Environmental and Host Risk Factors. In: Grob JJ, Stern RS, MacKie RM, Weinstock MA, ed. Epidemiology, causes and prevention of skin diseases. London: Blackwell, 1998: 235–42.
2 Rees JL, Flanagan N. Pigmentation, melanocortins and red hair. *Q J Med* 1999; **92:** 125–31.
3 Smith R, Healy E, Siddiqui S, Flanagan N, Steijlen PM, Rosdahl I, et al. Melanocortin 1 receptor variants in an Irish population. *J Invest Dermatol* 1998; **111:** 119–22.
4 Schioth HB, Phillips S, Rudzish R, Birch-Machin M, Wikberg J, Rees JL. Loss of function mutations of the human melanocortin 1 receptor are common and associated with red hair. *Biochem Biophys Res Commun* 1999; **260:** 488–91.

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Transcranial magnetic stimulation and auditory hallucinations in schizophrenia

Ralph E Hoffman, Nashaat N Boutros, Sylvia Hu, Robert M Berman, John H Krystal, Dennis S Charney

12 patients with schizophrenia and auditory hallucinations received 1 Hz transcranial magnetic stimulation of left temporoparietlal cortex. In a double-blind crossover trial, active stimulation significantly reduced hallucinations relative to sham stimulation.

Auditory hallucinations are reported by 50–70% of patients with schizophrenia and generally consist of spoken speech or voices. Response to drug treatment is often incomplete or non-existent, and these hallucinations can cause great distress, functional disability, and lack of behavioural control.

Silbersweig and colleagues[1] described regional brain activation by use of ^{15}O positron emission tomography when auditory hallucinations occurred in six patients with schizophrenia. Blood flow activation was detected in left temporoparietal auditory-linguistic association cortex and in thalamic, hippocampal, and striatal regions. Low frequency (1 Hz), extended duration (15–30 min), repetitive transcranial magnetic stimulation (rTMS) reduces activation in the brain area directly stimulated as well as in other functionally connected brain areas.[2,3] We postulated that low frequency rTMS delivered to the left temporoparietal cortex would curtail auditory hallucinations by reduction of excitability of distributed neurocircuitry that produce these experiences.

12 right-handed patients with auditory hallucinations who met Diagnostic and Statistical Manual IV (DSM-IV) diagnostic criteria for schizophrenia (eight paranoid type, four schizoaffective type; ten men; mean age 41·8 years [SD 8·6]) were included. Education level of the participants in grades was mean 14·2 (SD 1·8); a level of 14 grades corresponds to 2 years of college. All patients received antipsychotic drugs and were maintained on these drugs without change in dose throughout the study period. Five patients received concomitant anticonvulsant drugs (four valproate semisodium, one carbamazepine). All patients had daily auditory hallucinations without remission for at least 6 months. Auditory hallucinations were either continuous (three) or intermittent (nine). Each patient had normal routine laboratory studies, electrocardiogram, and electroencephalogram.

Motor threshold was identified as the minimum magnetic field strength required to produce left thenar muscle activation by single transcranial magnetic stimulation pulses delivered to the motor cortex, confirmed by electromyographic monitoring, for at least four of eight trials. Site and strength of the motor threshold was redefined each session. 1 Hz stimulation at 80% motor threshold was then given midway between the left temporal (T_3) and left parietal (P_3) electroencephalogram electrode sites on the basis of the international 10–20 electrode placement system. Sham

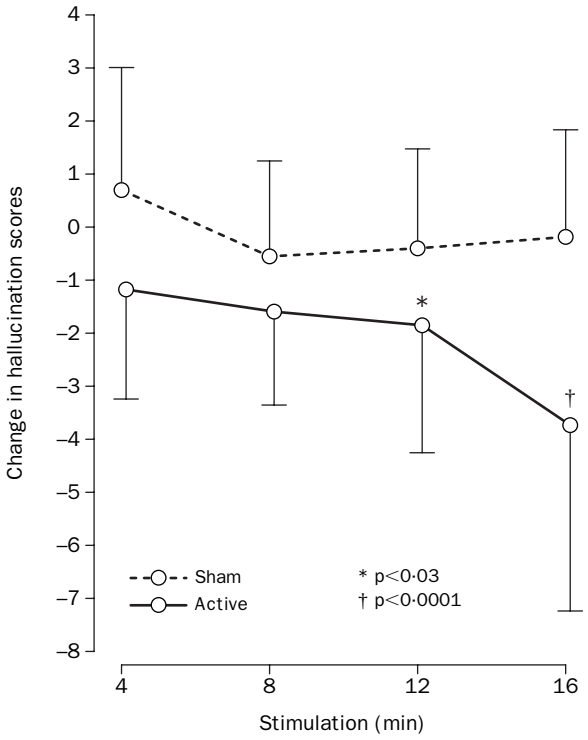

Figure 1: **Hallucination severity ratings and repetitive transcranial magnetic stimulation duration in active and sham stimulation trials**

Data analysed with a random effects model with Dunnett's criterion to adjust for multiple comparisons.

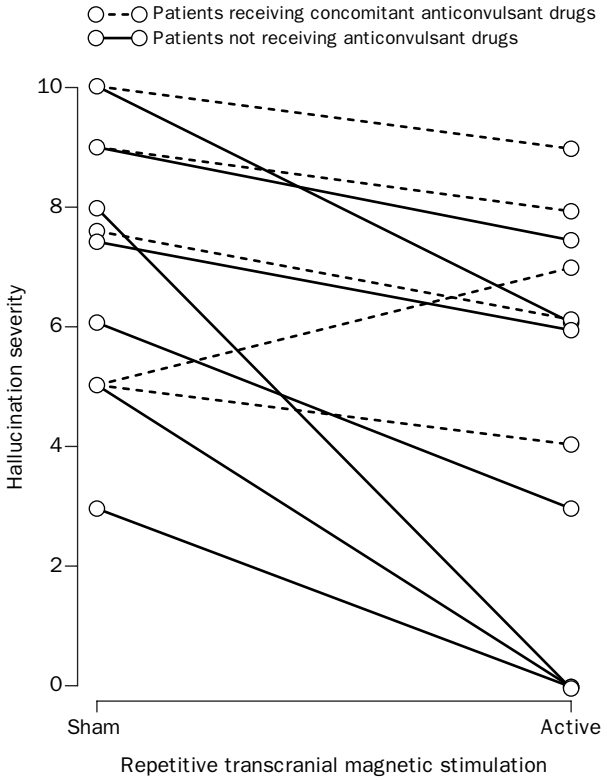

Figure 2: **Endpoint auditory hallucination severity for sham versus active repetitive transcranial magnetic stimulation**

stimulation was given at the same location, strength, and frequency with the coil angled 45° away from the skull to induce scalp stimulation but curtailing brain stimulation. A Cadwell magnetic stimulator system (Cadwell Inc, Kennewick, WA, USA) with a water-cooled, handheld figure-eight coil was used to deliver rTMS. Stimulation was initiated at 4 min for each condition and built up on successive days by 4 min to 16 min on day 4. Psychiatric symptoms were assessed daily with the Positive and Negative Symptom Scale (PANSS).

An earlier study showed that factors contributing to severity of auditory hallucinations varied between patients (for instance, frequency, loudness, content, number of voices, emotional distress, and level of distraction). Consequently, auditory hallucinations were assessed with an individualised, composite scale. A score of ten corresponded to a narrative description of the patient's hallucinations at the time of study entry, with zero corresponding to no hallucinations. For reassessments the patient produced a severity rating on the basis of these individualised anchor points. Higher scores were permitted if the patient's hallucination severity exceeded that at study initiation. Trials of active versus sham stimulation took place on separate weeks with 2–3 days separating each trial. Baseline hallucination assessments for each trial were done just before initiation of each stimulation condition and reflected the 24 h before. Reassessments took place the morning after each of the four rTMS sessions and indicated overall hallucination severity since the last rTMS session. Patients, clinical interviewers, and clinical staff were unaware of stimulation condition. Patients randomised to receive sham stimulation first received active stimulation the second week and viceversa.

Besides complaints of mild headache in two cases after active stimulation, patients tolerated rTMS without difficulty. Mean (SD) baseline hallucination score was 8·5 (2·2) for the active rTMS trial and 7·5 (2·6) for the sham rTMS trial. Symptom improvements relative to baseline were significant following 12 and 16 minutes of active stimulation but not for any duration of sham stimulation (figure 1). In all but one case, hallucination severity was lower after the active stimulation sequence than the sham stimulation sequence (figure 2). Endpoint hallucination ratings were analysed by use of a repeated measure ANOVA with two additional factors; order of stimulation (active or sham first), and concomitant treatment with anticonvulsant drugs. Reductions in hallucination severity after active compared with sham stimulation were significant ($p<0·006$), as was the interaction between change in hallucination severity and anticonvulsant drugs ($p<0·02$) showing reduced treatment effects with these drugs (figure 2). No effect of order of stimulation was seen.

Other positive symptoms and negative symptoms did not change much after rTMS. Follow-up assessments of the eight patients classified as responders (ie, hallucination severity improved for active relative to sham stimulation by score >1) indicated that auditory hallucinations returned roughly to baseline 1 day after the course of active rTMS in two patients, 4 days in two, 5 days in one, 2 weeks in one, 3 weeks, and 2 months in one. Left temporoparietal cortex, the site of rTMS in this study, is a brain area critical in perceiving spoken speech.[4] Our findings therefore support the hypothesis that speech perception neurocircuitry plays a part in the generation of hallucinated speech.

Not all patients showed robust improvements in hallucinations after active rTMS. One factor contributing to variable response was concurrent anticonvulsant drug treatment, which seemed to reduce rTMS effects. This observation suggests that higher levels of signal propagation are required for rTMS to curtail auditory hallucinations, or that symptoms prompting administration of anticonvulsant drugs (eg, mood lability) are negative predictors of rTMS response. Other factors that might contribute to the variability of rTMS effects include individual differences in anatomical location of speech processing areas,[5] variable location of cortical activation producing auditory hallucinations,[1] and differences in skull-brain relation, and baseline physiology.

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1 Silbersweig DA, Stern E, Frith C, et al. A functional neuroanatomy of hallucinations in schizophrenia. *Nature* 1995; **378:** 176–79.
2 Siebner HR, Tormos JM, Ceballos-Baumann AO, et al. Low-frequency repetitive transcranial magnetic stimulation of the motor cortex in writer's cramp. *Neurology* 1999; **52:** 529–37.
3 Wasserman EM, Wedegaertner FR, Ziemann U, et al. Crossed reduction of human motor cortex excitability by 1 Hz transcranial magnetic stimulation. *Neurosci Lett* 1998; **250:** 141–44.
4 Fiez JA, Raichle ME, Balota DA, et al. PET activation of posterior temporal regions during auditory word presentation and verb generation. *Cereb Cortex* 1996; **6:** 1–10.
5 Ojemann GA. Cortical organization of language. *J Neurosci* 1991; **11:** 2281–87.

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Circulating plasma platinum more than 10 years after cisplatin treatment for testicular cancer

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We have shown in patients cured from metastatic testicular cancer that up to 20 years after administration of cisplatin-containing chemotherapy, circulating platinum is still detectable in plasma. This finding may influence the development of long-term, treatment-related side-effects.

More than two decades after the introduction of cisplatin-containing chemotherapy for metastatic testicular cancer, this treatment remains one of the few with a high curative potential in disseminated cancer. The success of this therapy has resulted in increased survival in patients who have had testicular cancer. Therefore, the long-term sequelae of cytostatic treatment, such as cardiovascular problems or secondary malignancies, is becoming increasingly relevant.[1,2] The pathogenesis of the long-term sequelae of cisplatin combination chemotherapy in patients with testicular cancer has not yet been fully elucidated but it has been associated with prolonged retention of platinum in the body.

During a long-term follow-up investigation in 61 testicular-cancer patients cured with cisplatin combination chemotherapy more than 10 years previously, we measured plasma platinum concentrations using a sensitive assay. The median age of the patients at the time of chemotherapy was 27 years (range 17–36 years). The median age at the time of follow-up investigation was 42 years (30–50 years), with a median follow-up of 14 years (10–20 years). Patients were treated with four courses of cisplatin, bleomycin, and vinblastine or etoposide, every 3 weeks. 17 patients additionally received maintenance therapy with vinblastine and cisplatin for a maximum of 1 year. The total amount of administered cisplatin per patient ranged from 350 to 950 mg/m^2 (663–1846 mg). The 44 patients without maintenance chemotherapy received a mean cisplatin dose of 400 mg/m^2 (SD 14; range 350–450 mg/m^2 [663–987 mg]), and the 17 patients who also were treated with maintenance chemotherapy received a mean cisplatin dose of 801 mg/m^2 (SD 99, range 600–950 mg/m^2 [1191–1846]). Plasma platinum concentrations in these patients were compared with those of 20 control patients who were cured from stage I testicular cancer by orchidectomy without chemotherapy. The median age of the control patients at the time of orchidectomy was 26 years (18–38 years). The median age at follow-up was 42 years (30–50 years), with a median follow-up duration of 14 years after orchidectomy (10–20 years). Platinum concentrations were measured in masked plasma samples by a sensitive procedure during which high-pressure decomposition of samples is followed by an adsorptive voltammetric measurement.[3] The limit of quantification of platinum was 6 pg/g plasma. Measurements were done in duplicate; the coefficient of variation and day-to-day variation were 6% and 5%, respectively.

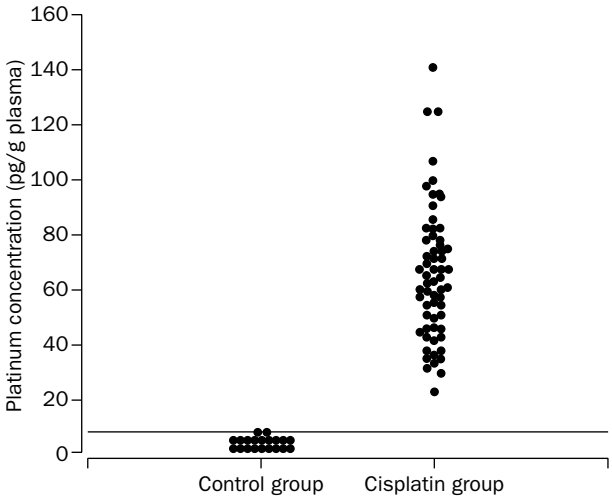

Figure 1: **Plasma platinum concentrations of 61 cured testicular cancer patients 10–20 years after cisplatin combination chemotherapy and 20 cured testicular cancer patients 10–20 years after orchidectomy**
Horizontal line=limit of quantification of platinum (6 pg/g plasma).

The platinum concentrations in the plasma of the 61 patients 10–20 years after cisplatin administration were significantly higher than those of the 20 control patients (cisplatin group: mean platinum concentration 64·9 pg/g plasma [SD 24·5] *vs* control group: 18 patients with platinum concentrations below the limit of detection and two patients with platinum concentrations at the limit of detection; Mann-Whitney *U* test, p<0·0001; figure 1). In all chemotherapy patients, the plasma platinum concentrations were above the limit of quantification, indicating that up to 20 years after

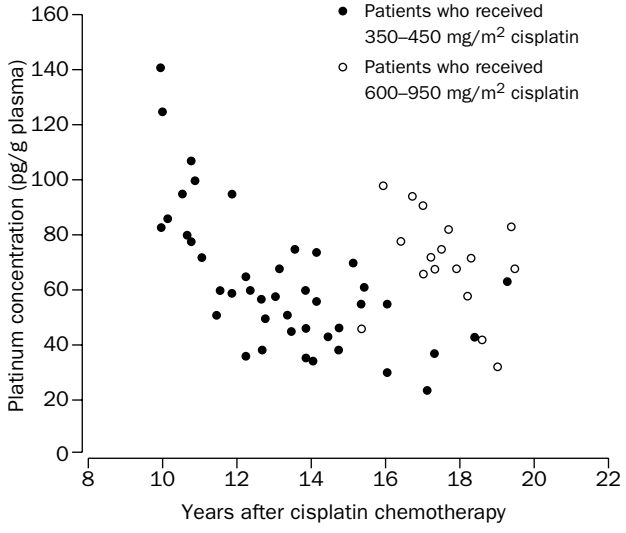

Figure 2: **Concentration-time plot of plasma platinum concentrations of 61 cured testicular-cancer patients 10–20 years after cisplatin combination chemotherapy**