*JID*Open

Germline Melanocortin-1-Receptor Genotype Is Associated with Severity of Cutaneous Phenotype in Congenital Melanocytic Nevi: A Role for MC1R in Human Fetal Development

Veronica A. Kinsler[1,2], Sayeda Abu-Amero[2], Peter Budd[3], Ian J. Jackson[3], Susan M. Ring[4], Kate Northstone[4], David J. Atherton[1], Neil W. Bulstrode[5], Philip Stanier[6], Raoul C. Hennekam[7], Neil J. Sebire[8], Gudrun E. Moore[2,10] and Eugene Healy[9,10]

Congenital melanocytic nevi (CMN) are pigmented birthmarks that affect up to 80% of the skin surface area. The increased frequency of CMN in families of severely affected individuals is suggestive of a predisposing germline genotype. We noted a high prevalence of red hair in affected families, and considered a role for *MC1R* in this condition. A cohort of 166 CMN subjects underwent pigmentary phenotyping, with *MC1R* genotyping in 113. Results were compared with a local control group of 60 unrelated children and with 300 UK children without CMN. CMN subjects had higher prevalences of red hair and a red-haired parent than local controls and had a higher rate of compound heterozygosity and homozygosity for *MC1R* variants. The presence of a V92M or R allele (D84E, R151C, R160W, D294H) was associated with increasing size of the CMN, implying a growth-promoting effect of these alleles. Unexpectedly, the V92M and R151C alleles were also strongly associated with birth weight in the CMN cohort, a finding confirmed in the control group. The effect of germline *MC1R* genotype on development and severity of CMN led us to investigate potential broader effects on growth, revealing a role for *MC1R* in normal fetal development.

INTRODUCTION

Congenital melanocytic nevi (CMN) are congenital moles that cover up to 80% of the body surface area and can number hundreds in a single individual. They are permanent and grow in proportion to the child, although they often lighten in color (Strauss and Newton Bishop, 2008; Kinsler

[1]Department of Paediatric Dermatology, Great Ormond Street Hospital for Children, London, UK; [2]Clinical and Medical Genetics Unit, Institute of Child Health, London, UK; [3]MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK; [4]School of Social and Community Medicine, University of Bristol, Bristol, UK; [5]Department of Plastic Surgery, Great Ormond Street Hospital for Children, London, UK; [6]Neural Development Unit, Institute of Child Health, London, UK; [7]Department of Paediatrics, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; [8]Department of Histopathology, Great Ormond Street Hospital for Children, London, UK and [9]Dermatopharmacology, University of Southampton, School of Medicine, Southampton, UK

[10]These authors contributed equally to this work.

Correspondence: Veronica A. Kinsler, Department of Paediatric Dermatology, Great Ormond Street Hospital for Children, London WC1N 3JH, UK. E-mail: v.kinsler@ucl.ac.uk; Eugene Healy, Dermatopharmacology, University of Southampton, School of Medicine, Southampton, UK. E-mail: e.healy@soton.ac.uk

Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; CMN, congenital melanocytic nevus or nevi; MC1R, melanocortin-1-receptor

Received 31 October 2011; revised 23 January 2012; accepted 13 February 2012; published online 10 May 2012

and Bulstrode, 2009; Kinsler *et al.*, 2009b). They are currently categorized by projected adult size (PAS) of the largest nevus and by total number of nevi (Ruiz-Maldonado, 2004). Neurological abnormalities such as neuromelanosis, congenital malformations, hydrocephalus, and benign tumors are the commonest complication of CMN, with the prevalence increasing with the severity of cutaneous lesions (Kadonaga and Frieden, 1991; DeDavid *et al.*, 1996; Kinsler *et al.*, 2008). Primary malignant melanoma of the skin or the CNS is a much rarer complication in childhood (Hale *et al.*, 2005; Krengel *et al.*, 2006; Kinsler *et al.*, 2009a), although almost universally fatal, and prophylactic removal of skin lesions has not been shown to decrease the risk of malignancy (Kinsler and Bulstrode, 2009). The recent finding of characteristic facies in children with CMN has broadened the description of this condition, and the term CMN syndrome has been proposed with preliminary diagnostic criteria (Kinsler *et al.*, Am J Med Genet, 2012 (in press)). The genetic cause of these syndromic features is likely to be a somatic change affecting the ectoderm or neural crest, but is still to be elucidated.
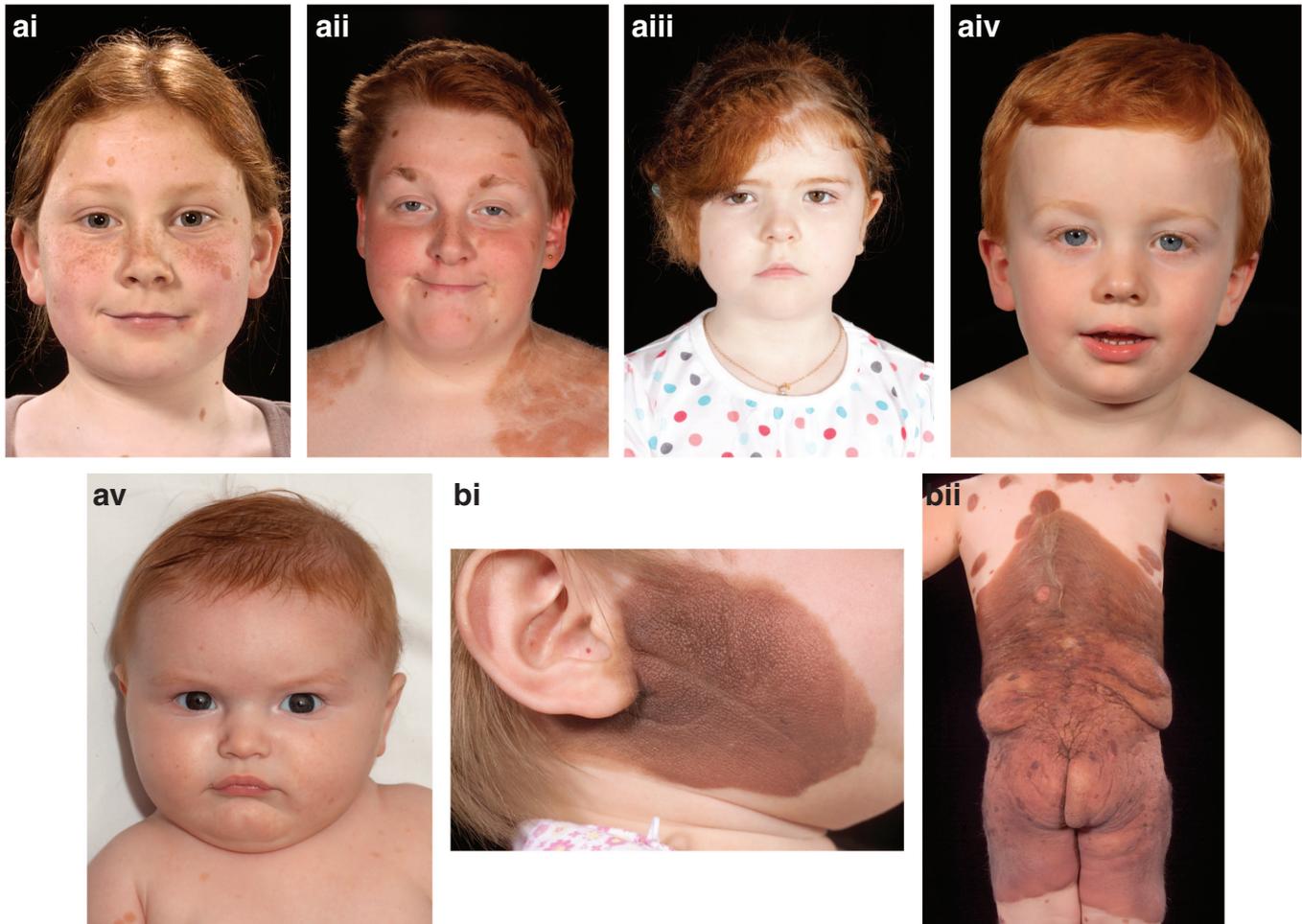
There is, however, evidence of a germline predisposition for the development of CMN syndrome; this includes a higher prevalence of smaller CMN in family members of affected individuals (Kinsler *et al.*, 2009a), together with a handful

Figure 1. **Clinical images of children with congenital melanocytic nevi (CMN).** Examples of (**a**i–v) red-haired children with CMN, and (**b**) different cutaneous phenotypes of CMN. (i) Projected adult size (PAS) 10–20 cm, (ii) PAS > 60 cm with several other nevi also visible. Written, informed consent was obtained for publication in all cases.

of reports of large CMN in first-degree family members (Danarti *et al.*, 2003; de Wijn *et al.*, 2010). We hypothesized that the melanocortin-1-receptor (*MC1R*) gene might have a role in this predisposition, on the basis of a clinical observation of a high prevalence of red hair in families of children with CMN (for examples see Figure 1). The red hair/fair skin/freckling phenotype is strongly associated with variants of *MC1R* on chromosome 16q24.3 (Valverde *et al.*, 1995; Box *et al.*, 1997; Schioth *et al.*, 1999; Healy *et al.*, 2000; Bastiaens *et al.*, 2001). MC1R is a G-protein-coupled receptor expressed on melanocytes, melanoma cells, and CMN cells (Ghanem *et al.*, 1988; Donatien *et al.*, 1992; Mountjoy *et al.*, 1992; Loir *et al.*, 1999). The gene is highly polymorphic, with over 100 variants reported to date. Variants with a stronger association with the red-hair phenotype in humans have been designated R alleles (D84E, R151C, R160W, and D294H), whereas those with a weaker association are designated r alleles (Sturm *et al.*, 2003). This study was undertaken to examine whether the *MC1R* genotype influenced the development of CMN.

RESULTS

A total of 166 children with CMN were included in the study. The cutaneous phenotype was classified as in Table 1.

Pigmentary phenotype data

Comparisons between affected and unaffected subjects were restricted to Caucasian subjects of Northern European ancestry (i.e., white-skinned individuals with four Northern European grandparents) who constituted the majority of the study group. Two control groups were ascertained; these were designated as control groups A (60 unrelated children from the same school, street, or locality as the affected individuals) and B (300 UK children without CMN from the Avon Longitudinal Study of Parents and Children (ALSPAC)) (Jones *et al.*, 2000; Golding *et al.*, 2001). Comparison of the pigmentary phenotype of the Northern European white children showed no statistical differences in phenotype between control groups A and B, although as the numbers in group A are relatively small we cannot exclude the possibility that these groups were different in some respect, and as a result we have used control group B for genotype

Table 1. Cutaneous phenotype of the CMN cohort

Projected adult size of largest CMN (cm)	N (%)	Site of largest CMN	N (%)
<10	35 (21)	Face	10 (6)
10–20	28 (17)	Scalp	19 (12)
20–40	27 (16)	Neck	85 (51)
40–60	24 (15)	Trunk	25 (15)
>60	48 (29)	Scalp/neck/trunk	9 (6)
No single larger lesion	4 (2)	Face/scalp	14 (8)
Missing data	0	No single larger lesion	4 (2)
		Missing data	
Total	166 (100)	Total	166 (100)

Total number of other nevi at birth	N (%)	Total number of other nevi at enrollment	N (%)
0	28 (17)	0	28 (17)
1–10	37 (22)	1–10	25 (15)
11–20	25 (15)	11–20	20 (12)
21–50	26 (16)	21–50	19 (11)
51–100	17 (10)	51–100	22 (13)
101–200	8 (5)	101–200	28 (17)
>200	3 (2)	>200	18 (11)
Missing data	22 (13)	Missing data	6 (4)
Total	166 (100)	Total	166 (100)

Abbreviation: CMN, congenital melanocytic nevi.

Table 2. Pigmentary phenotype of the CMN cohort and control groups

	CMN cohort (N=129)	Control group A (N=55)	Control group B (N=287)
Predominant hair color			
Blonde/light brown	83/119 (69.7)	46/53 (86.8)	230/287 (80.1)
Dark brown/black	25/119 (21.0)	4/53 (7.5)	49/287 (17.1)
Red	11/119 (9.3)	3/53 (5.7)	8/287 (2.8)
Peripheral predominant iris color			
Blue/gray	69/107 (64.5)	34/54 (63.0)	164/287 (57.2)
Green/hazel	12/107 (11.2)	10/54 (18.5)	69/287 (24.0)
Dark brown	26/107 (24.3)	10/54 (18.5)	54/287 (18.8)
Freckles	24/110 (21.8)	19/54 (35.2)	77/287 (26.8)

Abbreviation: CMN, congenital melanocytic nevi.
Numbers shown are absolute values with percentages in brackets.

data. There were significantly fewer blonde/light brown–haired individuals in the CMN cohort than in control group A and separately in control group B ($P=0.0215$ and $P=0.0275$ respectively; Table 2). In addition, fewer dark brown/black-haired individuals were present in the CMN cohort than in control group A ($P=0.0293$), whereas this was similar in the CMN cohort and control group B. Conversely, CMN children were more likely to have red hair than the larger control group B ($P=0.0085$), whereas this was not significantly different from the number of red-haired children in control group A. However, CMN children were more likely to have at least one red-haired parent (32 of 107 (30%)) than control group A (1 of 47 (2%)), respectively ($P<0.0001$), evenly divided between mothers and fathers. Parental pigmentary phenotype was not available for control group B. The pigmentary phenotype of the Northern European white children is shown in Table 2.

MC1R variants, particularly the R alleles, are known to be causally associated with red hair (Valverde *et al.*, 1995; Box *et al.*, 1997; Smith *et al.*, 1998; Sulem *et al.*, 2007), and there is some evidence that the *MC1R* genotype may be associated with blonde/light brown hair (Box *et al.*, 1997; Smith *et al.*, 1998). On the basis of these phenotypic observations between affected and unaffected groups, we hypothesized that the *MC1R* genotype may influence the development of CMN.

MC1R genotype data

According to the phenotyping described above, comparison of the *MC1R* gene between cohorts was restricted to white-skinned children of Northern European ancestry. To ensure sufficient numbers for statistical analysis of prevalence of *MC1R* polymorphisms, members of the affected group and control group B were sequenced for *MC1R*. Individuals with CMN were more likely to have two *MC1R* variant alleles than controls ($P=0.0052$, Table 3). More detailed analysis indicated that this was not due to a difference in the frequency of a particular *MC1R* variant between these two groups (see Supplementary table S1 online). The *MC1R* genotype in three pairs of affected and unaffected skin samples was identical with the germline genotypes, suggesting that somatic *MC1R* mosaicism was not the causative feature of affected versus unaffected skin.

In addition to coding region variants, a known SNP in the 3′ UTR (rs3212369) was found at a high frequency in both populations, and found to cosegregate strongly but not exclusively with the V92M and T314T alleles ($P<0.001$ for Pearson's correlation coefficients on all combinations of two variables). However, as an extension to previous reports (Harding *et al.*, 2000), the pattern of segregation suggested that this SNP arose independently of the T314T variant. This implies that either the V92M variant arose twice independently, on the 3′ UTR (rs3212369) and T314T alleles, or that there was intragenic recombination within *MC1R*.

Association between the MC1R genotype and CMN phenotype

In the CMN cohort, the presence of a V92M or an R allele (D84E, R151C, R160W, D294H) was found to be significantly associated with increasing projected adult size (PAS) of the largest CMN ($P=0.01$) (Figure 2). We examined this

Table 3. Comparison of prevalence of carriage of non-synonymous *MC1R* variants found in the white Northern European Caucasian children of the CMN cohort and control group B

	CMN cohort, *N* (%)	Control group B, *N* (%)	Fisher's exact *P*-value
Wild type	17/84 (20.2)	82/267 (30.7)	0.0711
Heterozygous	40/84 (47.6)	139/267 (52.1)	0.5320
Compound heterozygous	20/84 (23.8)	39/267 (14.6)	0.0647
Homozygous	7/84 (8.3)	7/267 (2.6)	0.0478*
Compound heterozygous or homozygous	27/84 (32.1)	46/267 (17.2)	0.0052**
Total	84/84 (100)	267/267 (100)	

Abbreviation: CMN, congenital melanocytic nevi.
Significance at 0.05 level* and **0.01 level.

Figure 2. **Association between *MC1R* genotype and severity of cutaneous phenotype in congenital melanocytic nevi (CMN).** Dot plot of proportions of V92M- or R allele–positive individuals with increasing projected adult size of largest CMN.

effect in more detail using >60 cm PAS as a cutoff, as this has previously been noted to be a significant predictor for increased incidence of malignant melanoma (Demenais *et al.*, 2010). The odds ratio for having a CMN of PAS >60 cm compared with those of <60 cm if carrying V92M or an R allele was 2.867 (95% CI 1.1257–6.537, $P = 0.012$).

Association between the *MC1R* genotype and family history of CMN

Thirty-four percent of the children with CMN had a first- or second-degree family history of CMN. If the *MC1R* genotype were one of the predisposing factors for development of CMN, a difference in *MC1R* genotype between those with and without a positive family history of CMN would be expected. This was indeed the case, with a significantly higher prevalence of at least one *MC1R* variant in those with a family history of CMN versus those in whom no first- or second-degree relative had a CMN (91 vs. 59% respectively, $P = 0.0062$). When confined to the V92M and/or R alleles, a similar preponderance of variants was noted in CMN children with a family history versus those without a family history of CMN (65 vs. 30%, $P = 0.018$).

Association of the *MC1R* genotype and birth weight

The above results, in which *MC1R* variants were associated with larger CMN and more nevi, indicate that the *MC1R* genotype influences the development of CMN. However, an additional possibility is the existence of a broader role for *MC1R* genotype in fetal growth and development, especially that *MC1R* variants function in promoting growth of the fetus. Therefore, we examined for associations between V92M and R alleles and birth weight in the CMN cohort and control group B. The presence of a V92M or R151C was significantly associated with increased birth weight in the CMN cohort (unstandardized coefficient +697 g, SE 216 g, $P = 0.002$), independent of gender and maternal smoking (Figure 3a). A similar significant effect of the same *MC1R* alleles was observed on birth weight (+187 g, SE 59 g, $P = 0.002$) in the
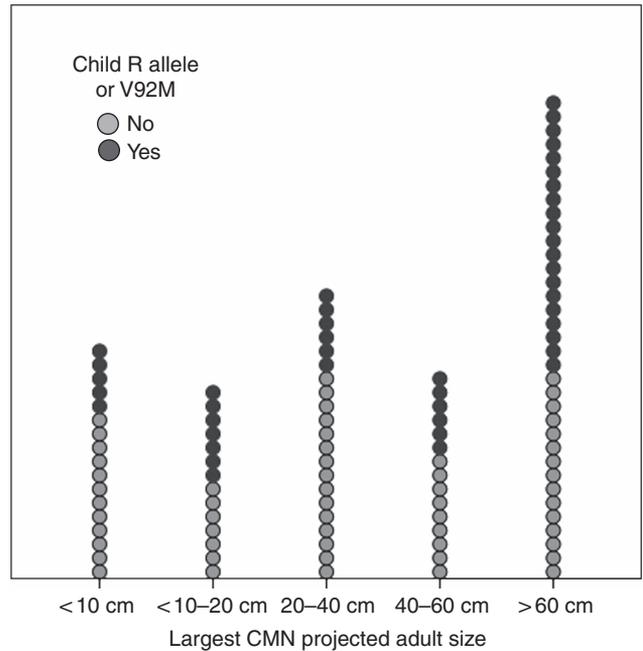
ALSPAC cohort, control group B (Figure 3b). This effect was independent of sex, gestation, maternal smoking, maternal weight pre-pregnancy, and maternal hypertension. Thus, the magnitude of this genotype effect is comparable to the effect of gender in this study. There was no significant association between R alleles as a group and birth weight in either cohort. No association between postnatal weight at the age of 49 months and *MC1R* genotype was found in control group B.

DISCUSSION

Previous studies of the effects of *MC1R* genotype in postnatal life have documented fewer acquired melanocytic nevi in red-haired children and adults (Duffy *et al.*, 2004; Dellavalle *et al.*, 2005), and in individuals homozygous or compound heterozygous for R alleles at the *MC1R* locus (Duffy *et al.*, 2004). In addition, genome-wide association studies have not found any association between *MC1R* genotype and numbers of acquired melanocytic nevi (Falchi *et al.*, 2006; Yeh and Bastian, 2009), and no association between *MC1R* variants and higher numbers of acquired nevi in families with inherited melanoma (Demenais *et al.*, 2010). However, in the current study, we have identified associations between families with CMN-affected children and the red hair pigmentary phenotype, and associations between *MC1R* variants and the presence of CMN, as well with more extensive CMN. Taken in the context of the effect on birth weight, this difference between congenital and acquired nevi may be due to the differential effects of MC1R during pre- and postnatal life, although other inherent genetic differences between congenital and acquired nevi could also be significant factors.
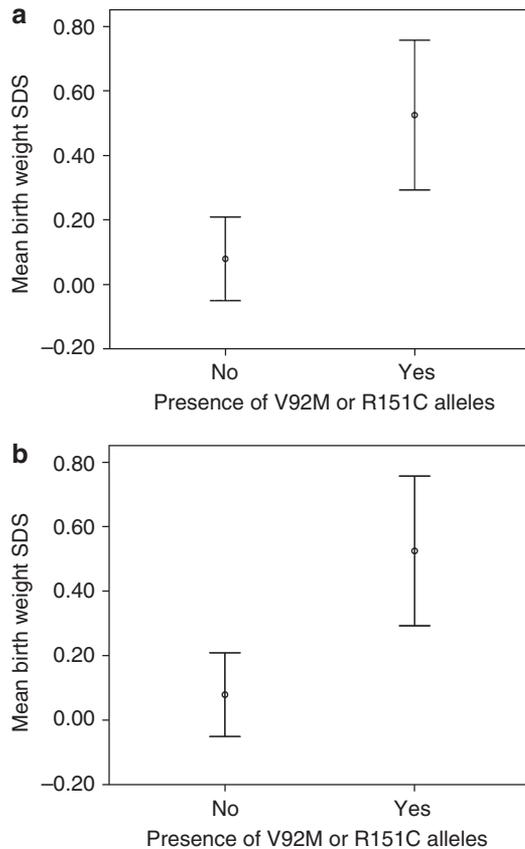
Figure 3. Association between *MC1R* genotype and birth weight. Effect of the presence of V92M or R151C alleles on birth-weight standard deviation score (SDS) in (**a**) the congenital melanocytic nevi (CMN) cohort, and (**b**) control group B. SDS includes the effects of gestation and sex, and a score of +1 is equivalent to an increase in one SD from the mean of the population. Error bars are 95% confidence intervals.

Our findings indicate that certain *MC1R* variants function to promote the growth of CMN during embryogenesis, and possibly the growth of the fetus as a whole. The exact mechanism whereby *MC1R* variants alter fetal growth and development are unknown, but might involve direct effects on the fetus and/or indirect effects through the presence of *MC1R* on trophoblast cells of the placenta (Thornwall *et al.*, 1997). MC1R is expressed on a wide range of adult human cell types including adipocytes (Hoch *et al.*, 2007; www.genecards.com; Rebhan *et al.*, 1997), supporting the increasing body of evidence that it has significant non-pigmentary roles (Robinson and Healy, 2002; Mogil *et al.*, 2005; Robinson *et al.*, 2010); alpha-MSH is involved in metabolic pathways, but this is currently understood to be via the other melanocortin receptors. Melanocortin-1-receptor (*MC1R*) genotype is known to interact with pigmentary and non-pigmentary pathways (Robinson and Healy, 2002; Cooper *et al.*, 2005; Robinson *et al.*, 2010), for example, altering the penetrance of other genes in melanoma such as *p53* (Patton *et al.*, 2005; Stefanaki *et al.*, 2007; Nan *et al.*, 2008) and *CDKN2A* (Box *et al.*, 2001; Debniak *et al.*, 2006). The latter effects are dependent not only on particular alleles but also on the number of variant alleles present (Demenais *et al.*,

2010). A similar interactive effect may be operating in individuals with CMN, with *MC1R* variants exerting a phenotype-exacerbating effect on a different somatic, or possibly germline, genetic alteration that affects CMN development. The effect of *MC1R* genotype on birth weight in our study was a generalized phenomenon that was found in two separate cohorts (CMN and ALSPAC), and in the *MC1R*-pigmentary context the previously reported interactive effects of *MC1R* genotype on oculocutaneous albinism (King *et al.*, 2003) was a generalized, rather than localized, effect on pigmentation. The fact that in the ALSPAC cohort of healthy children the larger birth weight in subjects with *MC1R* variants was not associated with any other dysmorphic features suggests that in the normal situation *MC1R* genotype may influence fetal development to affect body size in a proportionate manner. Interactive effects of *MC1R* with other genes affecting birth weight have not yet been investigated, and further work on this topic will be required in the future.

MC1R variants are a risk factor for melanoma development in the general population and are associated with increased risk of melanoma in *CDKN2A* mutation carriers (Box *et al.*, 2001; Demenais *et al.*, 2010). One important clinical consideration in our patient cohort is whether the pattern of germline *MC1R* genotype is responsible for the excess risk of malignant melanoma in individuals with CMN. The lifetime risk for melanoma is around 1–2% when all CMN are considered together as a group (Krengel *et al.*, 2006; Kinsler *et al.*, 2009a), which is similar in magnitude to the excess risk associated with *MC1R* variants in the normal population. The numbers of melanomas in our CMN cohort are too few to allow detailed investigations on the risk of melanoma attributable to *MC1R* variants, but it is likely that this genotype is at least contributing to melanoma risk. In a recent family-based melanoma study, the odds ratio for melanoma in *CDKN2A* mutation carriers was 5.67 (95% CI 2.1–15.29) for subjects with *MC1R* variants (Demenais *et al.*, 2010); thus, it is possible that the *MC1R* genotype may similarly alter the risk of melanoma in people with larger CMNs.

In conclusion, we have identified associations between CMN and red-hair/*MC1R* variants. Furthermore, the presence of a V92M or R allele was associated with larger CMN size and with larger birth weight in the CMN, and separately in the ALSPAC, groups consistent with a growth-promoting effect of these alleles during embryogenesis.

MATERIALS AND METHODS
This study was approved by the research ethics committee of Great Ormond Street Hospital for Children/Institute of Child Health, London. All aspects of this study comply with the Declaration of Helsinki Principles. Written, informed consent was given by parents in all cases, and by subjects, where appropriate.

CMN cohort
In all, 166 families with children with CMN were recruited for pigmentary phenotyping during clinical consultations, and offered *MC1R* genotyping. CMN phenotype was classified as previously published (Ruiz-Maldonado, 2004; Kinsler *et al.*, 2008, 2009a, 2009b) (Table 1; for examples see Figure 1). The total number of nevi at birth

was ascertained by history, direct examination, or photographs. Mean and median ages at enrollment to the study were 5.02 years (SEM 0.53) and 2.54 years (range 0.03–43.12), respectively. Follow-up during the study period was for a mean of 3.09 years and median of 3.12 years. Pigmentary phenotyping was performed by a single observer (VK) and was assessed after 1 year of age because of the changes frequently seen in hair and eye color in the first year. Hair color was classified using a chart of 12 colors; eye color was grouped into six standard colors, with peri-pupillary and predominant background iris color considered separately; freckling was recorded by grouped number and distribution; and skin type was assessed using the Fitzpatrick score. Pigmentary phenotype was obtained for 148 children, 143 mothers, and 130 fathers. Ancestry, up to the four grandparents, was also recorded; patients were classified as white Northern European Caucasians if their skin color was white and if all four of their grandparents were born in Northern Europe. Consent was obtained for *MC1R* genotyping in 113 families. Adequate blood samples for DNA extraction were obtained from 104 children, 78 mothers, and 58 fathers. With further written consent, three children had skin biopsies taken from affected and unaffected skin, whereas they were undergoing general anesthesia for other procedures.

Control group A

With the assistance of the CMN families, we recruited a geographically matched and sex-matched control population of individuals without CMN. Sixty families completed the same pigmentary phenotyping questionnaires as the CMN group, with telephone guidance from the same researcher. Fifty-five were white Northern European Caucasians, i.e., four grandparents born in Northern Europe; the mean and median age of these 55 children was 7.2 years (SEM 1.1) and 5.6 years (range 0.7–37.6), respectively. To check whether self-reported phenotypes were accurate, 25 CMN families (66 individuals) completed the pigmentary phenotype questionnaire before undergoing face-to-face phenotyping. Pearson's correlations for hair color and eye color were 0.509 and 0.855, respectively, significant at <0.05 level, supporting the use of self-reported questionnaires in this context.

Control group B

Ethical approval for this part of the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees. The children in this group born in 1992 had face-to-face pigmentary phenotyping by trained observers at the age of 49 months, and the presence of CMN was noted. The 300 samples were chosen randomly from those without CMN. Pigmentary phenotyping in the ALSPAC study was performed by trained research nurses as follows: hair color was compared with 11 real-hair swatches of measured luminosities, eye color was compared with six standard iris photographs, and skin reflectance was measured by spectrophotometer. *MC1R* genotyping was performed for all 300 samples, with 270 reliable results for Northern European Caucasian white children.

MC1R genotyping

DNA was extracted directly from all samples using standard extraction procedures. The 951 coding base pairs of the single exon of *MC1R*, as well as a further 416 base pairs of the 3′ UTR, were amplified by PCR as a single product and sequenced using four overlapping primer sets (5′-TCCAGCCAGGGAGAGGGTGTGA-3′ and 5′-AGCTGCAGGTGATCACGTCAAT-3′; 5′-CTCACCCATGTAC TGCTTCA-3′ and 5′-GAGTGTGAGATGCAGGAAGAAG-3′; 5′-CTC ATGGCCGTGCTGTACGT-3′ and 5′-CTTTGACAAACGGGGACCA G-3′; 5′-ACGCTCAAGGAGGTGCTGACAT-3′ and 5′-CCACCCAG AGACTTCACAT-3′).

Birth-weight analysis

In the CMN cohort, data on birth weight and maternal smoking were available for 128 children. Pre-term children were excluded on the basis that there might have been other abnormalities during the pregnancy that could confound any genotype effect. In control population B, data were available for birth weight, gestation, maternal smoking, maternal weight before pregnancy, and maternal hypertension for all children.

Statistical analysis

Comparisons of prevalence between groups (pigmentary characteristics and *MC1R* genotype) were made by Fisher's exact test. Analysis of the statistical effect of genotype on CMN phenotype and on birth weight was by ordinal and linear logistic regression analysis, respectively. For the *MC1R* genotype associations, we tested the following four variables initially against projected adult size of the main nevus: V92M, V60L, R163Q, and R alleles (grouped together, as they are known to be a clinically and physiologically cohesive group, but some are rare alleles); other alleles were excluded from this analysis as they were found in <5% of our population. To improve our model fit, we then combined the V92M and R allele groups to make the variable V92M or R allele. A Bonferroni correction was applied, reducing the level of significance from 0.05 to less than 0.0102062 (i.e., for a total of five statistical tests). We then used this combined variable, V92M or R allele, to test against birth weight in the CMN cohort, with a further refinement made to the model by using V92M combined with R151C (the commonest of the R alleles), to make the variable V92M or R151C. Again, with a Bonferroni correction (for two tests), this reduced the level of significance (*P*-value) from 0.05 to 0.0253206. For genotype testing against birth weight in the control group, we tested the single variable V92M or R151C.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Bastiaens M, ter Huurne J, Gruis N *et al.* (2001) The melanocortin-1-receptor gene is the major freckle gene. *Hum Mol Genet* 10:1701–8

Box NF, Duffy DL, Chen W *et al.* (2001) MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. *Am J Hum Genet* 69:765–73

Box NF, Wyeth JR, O'Gorman LE *et al.* (1997) Characterization of melanocyte stimulating hormone receptor variant alleles in twins with red hair. *Hum Mol Genet* 6:1891–7

Cooper A, Robinson SJ, Pickard C *et al.* (2005) Alpha-melanocyte-stimulating hormone suppresses antigen-induced lymphocyte proliferation in humans independently of melanocortin 1 receptor gene status. *J Immunol* 175:4806–13

Danarti R, Konig A, Happle R (2003) Large congenital melanocytic nevi may reflect paradominant inheritance implying allelic loss. *Eur J Dermatol* 13:430–2

Debniak T, Scott R, Masojc B *et al.* (2006) MC1R common variants, CDKN2A and their association with melanoma and breast cancer risk. *Int J Cancer* 119:2597–602

DeDavid M, Orlow SJ, Provost N *et al.* (1996) Neurocutaneous melanosis: clinical features of large congenital melanocytic nevi in patients with manifest central nervous system melanosis. *J Am Acad Dermatol* 35:529–38

Dellavalle RP, Johnson KR, Hester EJ *et al.* (2005) Children with red hair have more freckles but fewer melanocytic nevi: results from a cohort study of 280 three-year-olds. *Arch Dermatol* 141:1042–3

Demenais F, Mohamdi H, Chaudru V *et al.* (2010) Association of MC1R variants and host phenotypes with melanoma risk in CDKN2A mutation carriers: a GenoMEL study. *J Natl Cancer Inst* 102:1568–83

de Wijn RS, Zaal LH, Hennekam RC *et al.* (2010) Familial clustering of giant congenital melanocytic nevi. *J Plast Reconstr Aesthet Surg* 63:906–13

Donatien PD, Hunt G, Pieron C *et al.* (1992) The expression of functional MSH receptors on cultured human melanocytes. *Arch Dermatol Res* 284:424–6

Duffy DL, Box NF, Chen W *et al.* (2004) Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. *Hum Mol Genet* 13:447–61

Falchi M, Spector TD, Perks U *et al.* (2006) Genome-wide search for nevus density shows linkage to two melanoma loci on chromosome 9 and identifies a new QTL on 5q31 in an adult twin cohort. *Hum Mol Genet* 15:2975–9

Ghanem GE, Comunale G, Libert A *et al.* (1988) Evidence for alpha-melanocyte-stimulating hormone (alpha-MSH) receptors on human malignant melanoma cells. *Int J Cancer* 41:248–55

Golding J, Pembrey M, Jones R (2001) ALSPAC—the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol* 15:74–87

Hale EK, Stein J, Ben-Porat L *et al.* (2005) Association of melanoma and neurocutaneous melanocytosis with large congenital melanocytic naevi—results from the NYU-LCMN registry. *Br J Dermatol* 152:512–7

Harding RM, Healy E, Ray AJ *et al.* (2000) Evidence for variable selective pressures at MC1R. *Am J Hum Genet* 66:1351–61

Healy E, Flannagan N, Ray A *et al.* (2000) Melanocortin-1-receptor gene and sun sensitivity in individuals without red hair. *Lancet* 355:1072–3

Hoch M, Eberle AN, Wagner U *et al.* (2007) Expression and localization of melanocortin-1 receptor in human adipose tissues of severely obese patients. *Obesity* 15:40–9

Jones RW, Ring S, Tyfield L *et al.* (2000) A new human genetic resource: a DNA bank established as part of the Avon longitudinal study of pregnancy and childhood (ALSPAC). *Eur J Hum Genet* 8:653–60

Kadonaga JN, Frieden IJ (1991) Neurocutaneous melanosis: definition and review of the literature. *J Am Acad Dermatol* 24:747–55

King RA, Willaert RK, Schmidt RM *et al.* (2003) MC1R mutations modify the classic phenotype of oculocutaneous albinism type 2 (OCA2). *Am J Hum Genet* 73:638–45

Kinsler V, Bulstrode N (2009) The role of surgery in the management of congenital melanocytic naevi in children: a perspective from Great Ormond Street Hospital. *J Plast Reconstr Aesthet Surg* 62:595–601

Kinsler VA, Birley J, Atherton DJ (2009a) Great Ormond street hospital for children registry for congenital melanocytic naevi: prospective study 1988–2007. Part 1-epidemiology, phenotype and outcomes. *Br J Dermatol* 160:143–50

Kinsler VA, Birley J, Atherton DJ (2009b) Great Ormond street hospital for children registry for congenital melanocytic naevi: prospective study 1988–2007. Part 2—evaluation of treatments. *Br J Dermatol* 160:387–92

Kinsler VA, Chong WK, Aylett SE *et al.* (2008) Complications of congenital melanocytic naevi in children: analysis of 16 years' experience and clinical practice. *Br J Dermatol* 159:907–14

Krengel S, Hauschild A, Schafer T (2006) Melanoma risk in congenital melanocytic naevi: a systematic review. *Br J Dermatol* 155:1–8

Loir B, Perez SC, Ghanem G *et al.* (1999) Expression of the MC1 receptor gene in normal and malignant human melanocytes. A semiquantitative RT-PCR study. *Cell Mol Biol (Noisy-le-grand)* 45:1083–92

Mogil JS, Ritchie J, Smith SB *et al.* (2005) Melanocortin-1 receptor gene variants affect pain and mu-opioid analgesia in mice and humans. *J Med Genet* 42:583–7

Mountjoy KG, Robbins LS, Mortrud MT *et al.* (1992) The cloning of a family of genes that encode the melanocortin receptors. *Science* 257:1248–51

Nan H, Qureshi AA, Hunter DJ *et al.* (2008) Interaction between p53 codon 72 polymorphism and melanocortin 1 receptor variants on suntan response and cutaneous melanoma risk. *Br J Dermatol* 159:314–21

Patton EE, Widlund HR, Kutok JL *et al.* (2005) BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Curr Biol* 15:249–54

Rebhan M, Chalifa-Caspi V, Prilusky J *et al.* (1997) GeneCards: integrating information about genes, proteins and diseases. *Trends in Genetics* 13:163

Robinson S, Dixon S, August S *et al.* (2010) Protection against UVR Involves MC1R-mediated non-pigmentary and pigmentary mechanisms *in vivo*. *J Invest Dermatol* 130:1904–13

Robinson SJ, Healy E (2002) Human melanocortin 1 receptor (MC1R) gene variants alter melanoma cell growth and adhesion to extracellular matrix. *Oncogene* 21:8037–46

Ruiz-Maldonado R (2004) Measuring congenital melanocytic nevi. *Pediatr Dermatol* 21:178–9

Schioth HB, Phillips SR, Rudzish R *et al.* (1999) Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair. *Biochem Biophys Res Commun* 260:488–91

Smith R, Healy E, Siddiqui S *et al.* (1998) Melanocortin 1 receptor variants in an Irish population. *J Invest Dermatol* 111:119–22

Stefanaki I, Stratigos AJ, Dimisianos G *et al.* (2007) p53 codon 72 Pro homozygosity increases the risk of cutaneous melanoma in individuals with dark skin complexion and among noncarriers of melanocortin 1 receptor red hair variants. *Br J Dermatol* 156:357–62

Strauss RM, Newton Bishop JA (2008) Spontaneous involution of congenital melanocytic nevi of the scalp. *J Am Acad Dermatol* 58:508–11

Sturm RA, Duffy DL, Box NF *et al.* (2003) The role of melanocortin-1 receptor polymorphism in skin cancer risk phenotypes. *Pigment Cell Res* 16:266–72

Sulem P, Gudbjartsson DF, Stacey SN *et al.* (2007) Genetic determinants of hair, eye and skin pigmentation in Europeans. *Nat Genet* 39:1443–52

Thornwall M, Dimitriou A, Xu X *et al.* (1997) Immunohistochemical detection of the melanocortin 1 receptor in human testis, ovary and placenta using specific monoclonal antibody. *Horm Res* 48:215–8

Valverde P, Healy E, Jackson I *et al.* (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat Genet* 11:328–30

Yeh I, Bastian BC (2009) Genome-wide associations studies for melanoma and nevi. *Pigment Cell Melanoma Res* 22:527–8