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2p15-p16.1 microdeletions encompassing and proximal to *BCL11A* are associated with elevated HbF in addition to neurologic impairment

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KEY POINTS

- Elevation of HbF in three patients heterozygous for distinct 2p15-p16.1 syndrome microdeletions affecting *BCL11A*
- Identification of novel, putative regulatory elements downstream of *BCL11A* that govern its expression in erythroid cells

ABSTRACT

Elevated fetal hemoglobin (HbF) ameliorates the clinical severity of hemoglobinopathies such as β-thalassemia and sickle cell anemia. Currently, the only curative approach for individuals under chronic transfusion/chelation support therapy is allogeneic stem cell transplantation. However, recent analyses of heritable variations in HbF levels have provided new therapeutic targets for HbF reactivation, chiefly, the transcriptional repressor BCL11A. Erythroid-specific BCL11A abrogation is now actively being sought as a therapeutic avenue, but the specific impact of such disruption in humans remains to be determined. While single nucleotide polymorphisms in *BCL11A* erythroid regulatory elements have been reported, coding mutations are scarcer. It is thus of great interest that patients have recently been described with microdeletions encompassing *BCL11A*. These patients display neurodevelopmental abnormalities, but whether they show increased HbF has not been reported. We have examined the hematological phenotype, HbF levels and erythroid *BCL11A* expression in 3 such patients. Haploinsufficiency of *BCL11A* induces only partial developmental γ-globin silencing. Of greater interest however, is that a patient with a downstream deletion exhibits reduced *BCL11A* expression and increased HbF. Novel erythroid-specific regulatory elements in this region may be required for normal erythroid *BCL11A* expression, whilst loss of separate elements in the developing brain may explain the neurological phenotype.

INTRODUCTION

Chromosome 2p15-p16.1 microdeletions constitute a contiguous gene deletion syndrome characterized by shared phenotypic traits including intellectual disability, growth retardation and distinct dysmorphisms. To date, 11 microdeletions of discrete lengths have been described, each associated with overlapping subsets of phenotypic features.[1] These deletions collectively encompass approximately 17 protein-coding genes and are accordingly associated with a complex spectrum of physical and mental traits. The smallest microdeletion yet has recently been reported to solely contain the *BCL11A* gene and is associated with hypotonia, mild intellectual delay, speech disorder, and gross motor impairments.[1]

BCL11A is a transcription factor that is highly expressed in the brain, B-lymphocytes and the adult erythroid lineage. It has emerged from several GWAS as a negative modulator of fetal hemoglobin expression.[2-4] Indeed, *BCL11A* knockout disrupts developmental silencing of human fetal γ-globin in transgenic mice.[5] Moreover, BCL11A knockdown in primary human erythroid cells results in increased HbF,[6] whilst conditional knockout in adult sickle cell disease transgenic mice leads to reactivation of *γ-globin* and amelioration of symptoms.[7] Erythroid-specific abrogation of BCL11A has thus been pursued as an attractive therapeutic for β-hemoglobinopathies.[8] While HbF-associated SNPs have been shown to disrupt an erythroid enhancer in the second intron of *BCL11A*, until recently, coding *BCL11A* mutations had not been reported.*[9-11] Here we have investigated the hematopoietic parameters of three patients with distinct *de novo*, heterozygous deletions encompassing, or proximal to, *BCL11A*. All display modestly reduced *BCL11A* expression and considerable HbF elevation. In one patient, a downstream deletion that leaves the *BCL11A* coding gene intact results in reduced *BCL11A* transcripts in erythroblasts, alluding to the existence of novel, erythroid regulatory elements within this region.

METHODS

See Supplemental Data.

RESULTS AND DISCUSSION

The three patients herein have been described previously.[12,13] The first is a 15-year old female with a 3.5 Mb deletion downstream of *BCL11A* (Figure 1A).[13] The second and third are an 8-year old female and a 7-year old male with 642 kb and 2.5 Mb deletions respectively covering the entire *BCL11A* gene (Figure 1A).[12] Although their neurological and physical traits have been extensively characterized, their hematopoietic profiles have not been previously assessed.

We first determined the extent to which these heterozygous deletions influence *BCL11A* expression in erythroblasts derived from the three patients. In all cases, *BCL11A* transcripts were significantly reduced by approximately 2-fold relative to parental expression (Figure 1B). We also specifically analyzed the abundance of different *BCL11A* mRNA isoforms, and found that those implicated in γ-globin repression (*BCL11A-XL* and *–L*)[5,6] were significantly down-regulated in the patients' erythroblasts, whilst short transcript variants (*BCL11A-S*) were not (Figure 1B). Expression of *KLF1* and *GATA1*, erythroid transcription factors that regulate globin expression, was unaltered (*data not shown*).

Complete blood counts revealed that hematological parameters were largely normal in the three individuals (Table S1). The third patient displayed mildly reduced haematocrit and increased variability in red blood cell size at 5 months of age. These findings were recapitulated in a follow-up count at 7 years of age. This patient also exhibited modest lymphocytosis at this age. BCL11A is highly expressed in B-cells and has been implicated in human B-lymphopoiesis.[7,14] It should be noted however that the first two patients showed normal lymphocyte levels, suggesting that these hypomorphic *BCL11A* mutations have minimal impact on B-cell numbers.

We next assayed relative amounts of hemoglobin isoforms by HPLC. All three patients had markedly elevated fetal γ-globin compared to their unaffected parents (Figures 1C and S1A). This was reflected both as a fraction of total fetal plus adult β-like globin, as well as α-globin (Figure S1B, C). HPLC analysis of hemoglobin tetramers determined the patients' HbF levels to be 7.3%, 4.8% and 6.2% respectively, compared to that of their parents (0.5%; 0.7% and 0.2%; 0.2% and 0.6%) (Figure 1C). Quantification of individual globin chains revealed that fetal γ-globin represented 11.5%, 8.2% and 10.0% of total β-like globin in the three patients, compared to parental levels of 1.5%; 1.9% and 1.3%; 1.9% and 2.0% (Figure S1A). In all individuals, a balanced ratio of β-like globin to α-globin was sustained (Figure S1D).

These results demonstrate that *BCL11A* haploinsufficiency has an appreciable effect on HbF expression. This mirrors findings of the master erythroid regulator KLF1, an upstream activator of *BCL11A*.[15,16] In the short time since a nonsense mutation in KLF1 was initially linked to hereditary persistence of fetal hemoglobin,[15] a raft of additional mutations in the locus have been described owing to focussed sequencing efforts,[17-20] culminating in the recent discovery of a KLF1 null individual.[21] Thus far, *BCL11A* coding mutations have not been frequently encountered. Given its implicated B-lymphopoietic and neurological roles, it is possible that *BCL11A* mutations with erythroid-restricted consequences (i.e. elevated HbF) will largely lie in regulatory elements with erythroid activity (as in [9]).

The approximately 2-fold down-regulation of *BCL11A* mRNA in Patients 2 and 3 can likely be attributed to monoalleleic expression arising from *BCL11A* heterozygosity. However, in the first patient, both *BCL11A* alleles are intact (Figure 1A). This raises the possibility that downstream regulatory elements within the deleted region are required for full erythroid expression of *BCL11A*. Inspection of this region revealed several prominent DNase-hypersensitive sites in adult erythroid cells that are co-bound by various combinations of key erythroid transcription factors (GATA1, TAL1, KLF1 and NFE2) and are enriched for the active enhancer-associated marker H3K4me1 (Figure 2).

These elements represent potential erythroid enhancers, although their functional characterization remains to be determined. It is also plausible that the deletion in Patient 1 might result in reduced *BCL11A* expression through alternative molecular mechanisms, such as downstream heterochromatic regions being brought into the vicinity of *BCL11A*.

The extent to which BCL11A need be disrupted to induce clinically protective HbF levels in sickle cell and thalassemic sufferers is unknown. The patients here exhibit approximately 5-10% HbF and 2-fold downregulated *BCL11A* mRNA. In sickle cell sufferers, such HbF levels are associated with reduced major organ failure, but higher amounts (>20%) are required to deter recurrent clinical crises.[22] Further, due to limited availability of tissue samples, we have been unable to ascertain whether the increased HbF in these patients is pancellular or heterocellular. This is an important consideration, as pancellular upregulation is predominantly associated with clinical amelioration. Indeed, studies using transgenic, humanized sickle cell mice found that HbF levels of approximately 10% or greater were clinically beneficial when expressed in at least two-thirds of peripheral erythrocytes[23] or higher.[24]

Lastly, it is worth noting that several of the genes deleted in the three patients are expressed during erythroid differentiation (Figure 1A), and their deletion could thus conceivably contribute to the elevated HbF observed. However, the striking phenotypic similarities of the patients (in terms of both *BCL11A* and HbF levels) might suggest that the HbF modifier effects of these genes, which are variably deleted in different combinations, are minimal.

Taken together, these findings suggest that modest down-regulation of erythroid BCL11A expression results in markedly increased HbF, supporting the utility of erythroid-specific disruption of BCL11A as a therapeutic for β-hemoglobinopathies. The fact that these hypomorphic *BCL11A* mutations are associated with a spectrum of neurodevelopmental defects further emphasizes the need for such abrogation to be erythroid-specific. We suggest that candidate erythroid regulatory elements, such as those described previously[9] and here, should be inspected during routine screening of HbF modifiers, and may represent targets for therapeutic intervention.

FOOTNOTE

*During the revision of this manuscript, an additional 3 patients were described[11], each with heterozygous 2p15-p16.1 deletions distinct from those reported here. Each of these patients exhibited a similar reduction in *BCL11A* expression to the cases here, as well as persistence of fetal hemoglobin and unperturbed lymphocytes. However, the reported patients exhibited markedly higher levels of HbF (16.1-29.7%) than those described here. The reason for this discrepancy is unclear, but may be due to modifiers, genetic or otherwise, that influence ethnic or regional variation in HbF levels.

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AUTHORSHIP CONTRIBUTIONS

APWF analyzed data and performed the bioinformatics. PP, VO, MP, AG, LV and AM provided important hematologic data. MM, FC, LV and FM performed cell culture and analyzed *BCL11A* mRNA expression levels. SS-S conducted the HPLC analysis. TP, ARM and JAS oversaw project design. TP and ARM conceived the study and contributed equally to this work. APWF, ARM and TP wrote the manuscript.

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DISCLOSURE OF CONFLICTS OF INTEREST

The authors have no conflict of interest to disclose.

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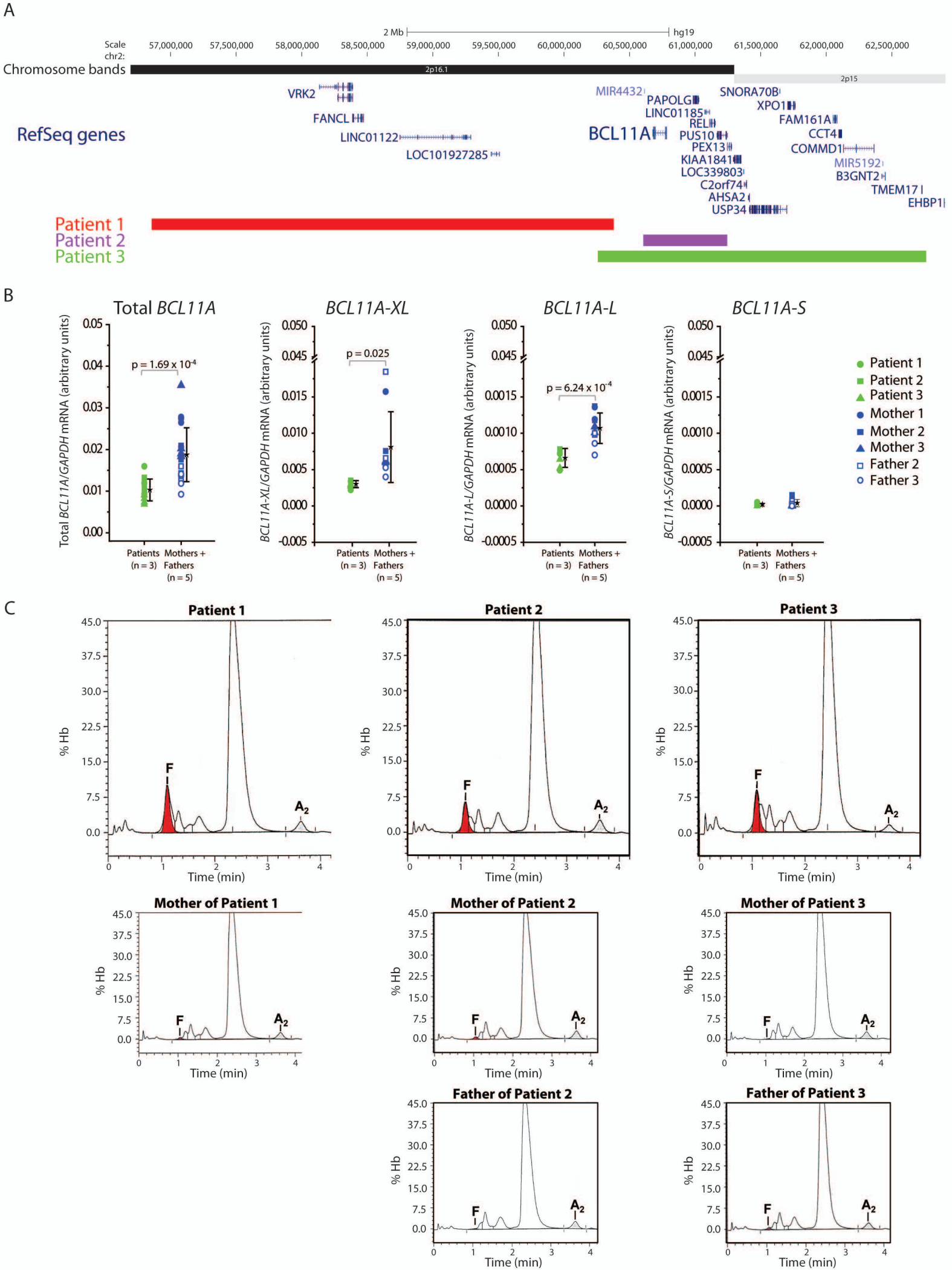
FIGURE LEGENDS

Figure 1. Patients with 2p15-16.1 microdeletions display depleted *BCL11A* expression in erythroblasts and markedly increased fetal γ-globin in peripheral blood. (A) Schematic of the 2p15-16.1 region showing segments that are deleted in the three patients. In addition to *BCL11A*, the following deleted genes are expressed at one or more stages during erythroid maturation[25]: VRK2, *FANCL*, *PAPOLG, REL*, *PUS10*, *PEX13*, *KIAA1841*, *C2orf74*, *AHSA2*, *USP34*, *XPO1*, *FAM161A*, *CCT4*, *COMMD1* and *B3GNT2*. (B) Real time RT-PCR quantification of *BCL11A* mRNA isoforms in erythroblasts expanded *in vivo* derived from patients (n = 3) and matched parents (n = 5). Shown are total *BCL11A* transcripts, isoforms previously implicated in γ-globin repression (*BCL11A-XL* and *–L*) and short variants (*BCL11A-S*). All levels have been normalized to *GAPDH* mRNA. Error bars represent standard deviation and *p* values signify ANOVA comparison. (C) HPLC determination of hemoglobin tetramer levels for the three patients and their parents. HbF peaks are shown in red.

Note that HbA_2 ($\alpha_2\delta_2$) levels are relatively constant across all samples (2.3%, 2.6% and 1.6% for the patients, and 2.2%; 2.8% and 2.6%; 2.4% and 2.2% respectively for the parents).

Figure 2. A downstream microdeletion associated with reduced *BCL11A* expression contains several putative erythroid enhancers. The region deleted in Patient 1 is shown in red. Shown are DNase-Seq tracks for adult erythroblasts (derived from peripheral blood $CD34^+$ cells), other hematopoietic cells in which BCL11A is expressed (B-cells) or not expressed (T-cells), and the developing fetal brain. Sequencing tracks have been emboldened for clarity. Potential erythroid regulatory elements are highlighted, and are bound in human pro-erythroblasts by different combinations of noted erythroid transcription factors (GATA1, TAL1, KLF1 and NFE2) as evidenced by ChIP-Seq. These regions are also marked by H3K4me1, typically associated with active enhancer elements. Of note, regions highlighted in pink display erythroid DNase hypersensitivity that is not present in the other hematopoietic cells, suggesting that they are erythroid-specific sites. Regions in gray signify DNase hypersensitive sites, at an alternative *VRK2* promoter and upstream element, which are present in other tissues. The segment in blue indicates a known erythroid enhancer in *BCL11A* intron 2, to which SNPs associated with HbF levels are localized.[9] A second cluster of high HbF-associated SNPs (marked by an asterisk) lies downstream of *BCL11A* but outside the deleted region. The fetal brain exhibits widespread DNase hypersensitivity across the deleted region which might account for the neurological phenotype of this patient.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Chromosome bands
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LINC01122
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SNORA70B
XPO1
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BCL11A-S/GAPDH mRNA (arbitrary units)
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p = 0.025
p = 6.24 x 10-4
Patients (n = 3)
Mothers + Fathers (n = 5)
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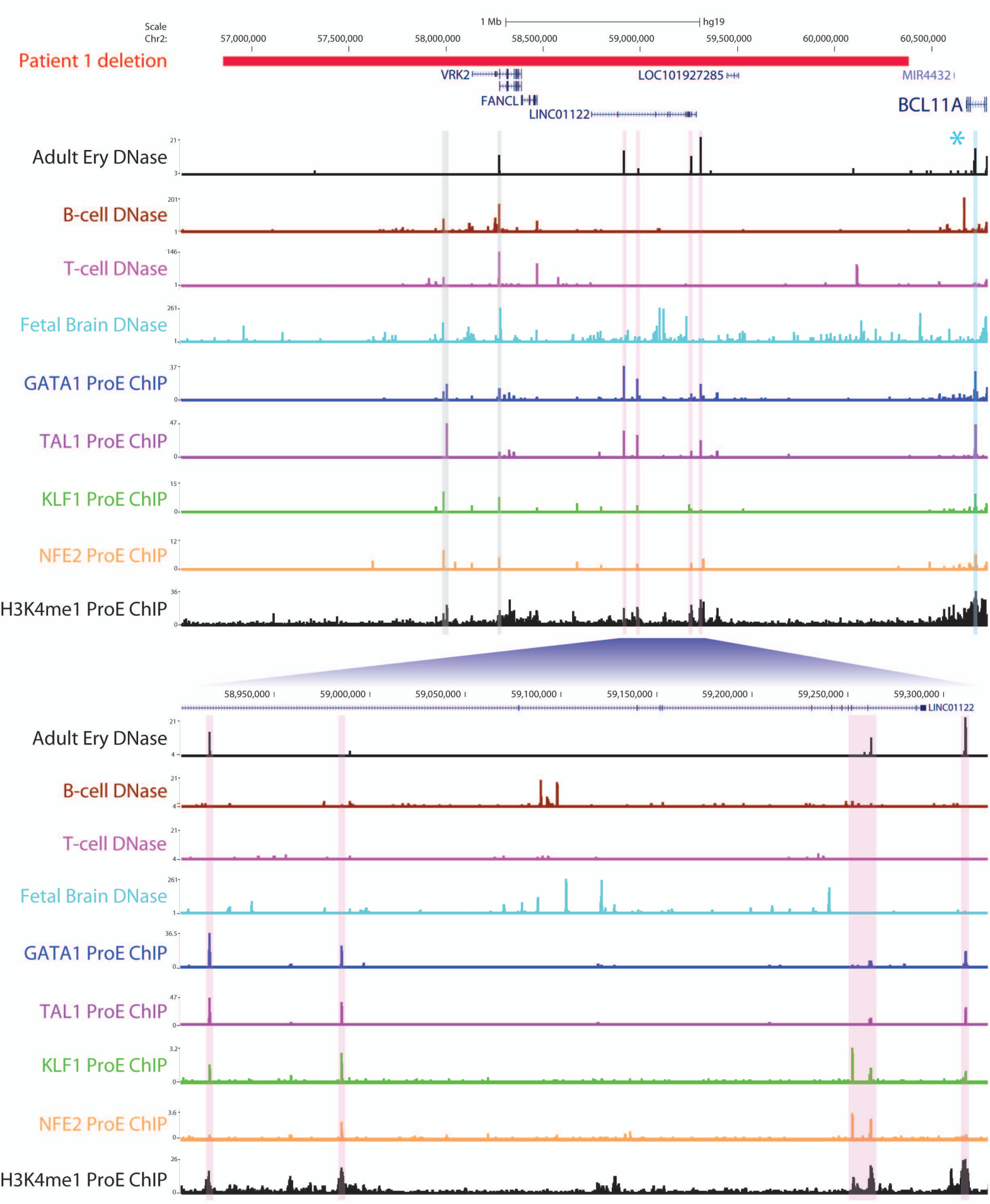
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Patient 1 deletion
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LINC01122
BCL11A
Adult Ery DNase
B-cell DNase
T-cell DNase
Fetal Brain DNase
GATA1 ProE ChIP
TAL1 ProE ChIP
KLF1 ProE ChIP
NFE2 ProE ChIP
H3K4me1 ProE ChIP
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