

ARTICLE

Deletions of 2q14 that include the homeobox engrailed 1 (EN1) transcription factor are compatible with a normal phenotype

John CK Barber^{*,1,2,3}, Viv K Maloney², Beverley Bewes⁴ and Emma Wakeling⁴

¹Wessex Regional Genetics Laboratory, Salisbury Health Care NHS Trust, Salisbury District Hospital, Salisbury, UK; ²National Genetics Reference Laboratory (Wessex), Salisbury Health Care NHS Trust, Salisbury District Hospital, Salisbury, UK; ³Human Genetics Division, Southampton University Hospitals Trust, Southampton, UK; ⁴NW Thames Regional Genetics Service, Kennedy Galton Centre, Northwick Park and St Mark's Hospitals, Harrow, UK

A novel transmitted 2–3 Mb deletion of 2q14.1–q14.2 was found in an affected boy from a consanguineous family with a possible diagnosis of PEHO syndrome (OMIM 260565). BAC FISH showed that the deletion included a minimum of 20 genes including the homeobox engrailed 1 gene (*EN1*). However, the same deletion was also found in his phenotypically normal father and brother (family 1). The phenotype of the proband may, therefore, have been coincidental to the deletion, a result of a recessive condition within or outside the deleted segment or possibly due to variable dosage compensation of *EN1* by the paralogous *EN2* gene at 7q36. BAC FISH also showed that this deletion overlapped with a previously reported transmitted deletion of 2q13–q14.1 that had no phenotypic consequences (family 2). The deleted regions contained a total of 32 genes and comprise the final 5.25 Mb of the ancestral chromosome 2B from which chromosome 2 was formed in man. These families provide further evidence that heterozygous deletions of regions of low gene density are compatible with a normal phenotype.

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Introduction

A growing number of relatively small imbalances are being detected using high resolution conventional cytogenetics, high-resolution comparative genomic hybridisation (HR-CGH)¹ and array CGH.^{2–5} Over 10% of the abnormalities detected using HR-CGH¹ and over 40% of those detected using array CGH^{2–5} are directly transmitted from parents to children. In a recent review, 130 families with directly transmitted unbalanced chromosome abnormalities were identified⁶ and, in the majority of these (77/130), both

proband and parents were phenotypically affected. However, in 30/130, an unaffected parent had the same imbalance as an affected child and, in 23/130, both the probands and parents were phenotypically normal. Here, we present further examples of an affected boy with the same 2–3 Mb deletion of 2q14.1–q14.2 as his unaffected father and brother. This deletion overlapped with a previous transmitted deletion of 2q13–q14.1 in a phenotypically normal mother and daughter⁷ which has now been mapped in more detail.

*Correspondence: Dr JCK Barber, Wessex Regional Genetics Laboratory, Salisbury Health Care NHS Trust, Salisbury District Hospital, Salisbury SP2 8BJ, UK. Tel: +44 1722 429080; Fax: +44 1722 338095; E-mail: john.barber@salisbury.nhs.uk
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Methods

G-banded chromosomes were analysed at the 550 or higher band level and FISH was carried out with 1 Megabase (Mb) clones or tiling path BACs selected from the Ensembl

database (www.ensembl.org/Homo_sapiens/cytoview). Karyotype results include the probes informative for the breakpoints only. Genes assigned to the deleted regions were obtained from the Ensembl database (v32) using the MartView data export tool (www.ensembl.org/Multi/martview). Messenger RNA homologies were obtained using the NCBI bl2seq alignment tool (www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgiBLASTN).

Family report and results

Family 1 del(2)(q14.1q14.2)

The proband was the sixth child born to consanguineous Pakistani parents. His mother had no history of miscarriages. The proband presented at birth with a left congenitally dislocated hip and minor dysmorphic features including long nails, overlapping fingers and toes and an unusual right ear with an overfolded helix and double ear lobe creases. At the age of 14 months, he had profound developmental delay, seizures, head circumference just below the 0.4th centile, marked hypertonia, puffy hands and feet and roving eye movements with hypoplastic optic discs. MRI scan of his brain showed simplified gyral

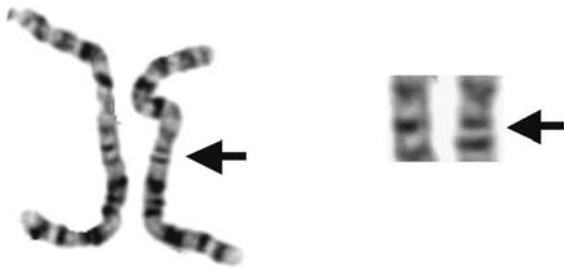


Figure 1 Partial karyotype of the deletion in family 1 with the deleted region indicated by black arrows. Note the reduction in the intensity of band 2q14.1 and the reduction in size of band 2q14.2 on the right hand chromosome 2 (and the magnified image of the same chromosome).

formation and a slightly small cerebellum. A diagnosis of progressive encephalopathy with oedema, hypsarrhythmia and optic atrophy (PEHO) syndrome (OMIM 260565) has been suggested.

One sister had died previously in infancy with a history of Dandy Walker malformation, profound developmental delay, brisk reflexes, roving eye movements and bilateral congenital dislocation of the hips. Although the CT scan findings were reportedly different to those of the proband, it is possible that both affected children had the same condition.

A deletion of 2q14.1 to 2q14.2 was found in the affected proband as well as his phenotypically normal father and 16-year-old brother (Figure 1). The brother attends mainstream school and is doing well academically. The father drives a minibus and has no history of learning difficulties at school. Two further brothers (aged 8 and 18) had normal chromosomes. The karyotype of the affected sister was reported as normal at the 400–550 band level of resolution but this may not have been sufficient to detect the subtle deletion of 2q and the slides were no longer available for retrospective review.

Using FISH on the proband, three clones from 2q14.1 and 2q14.2 were deleted (Figure 2) and all other probes gave normal results (Table 1). The proximal breakpoint was within the ~0.7 Mb region between 252I18 and 59P4 and the distal breakpoint within or close to the 2 kb overlapping region of 19E11 and 77A13. The karyotype was therefore 46,XY,del(2)(q14.1q14.2).ish del(2)(q14.1q14.2)(252I18+,59P4-,19E11-,77A13+). FISH with probes from opposite ends of the deletion confirmed that the deletion in his father and brother were of the same extent. The deletions had a minimum size of 2.24 Mb and a maximum size of 2.91 Mb. The minimal deleted region contains five known and 15 novel genes (Table 2) including the *EN1* homeobox engrailed 1 gene, which is 119.3 Mb from the telomere and maps to the proximal end of the deleted BAC 19E11 (119.3–119.4 Mb).

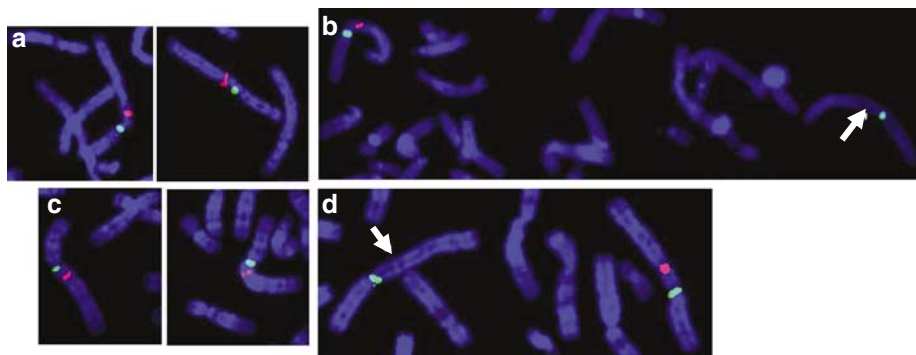


Figure 2 (a–d) Dual colour BAC FISH images of the deletions in families 1 and 2; normal red (TRITC) signals with (a) 395L14 and the absent signals with (b) 432G15 from the mother of family 1; normal signals with (c) 19E11 and absent signals with (d) 59P4 from the father of family 2. White arrows indicate the position of the missing signals and the green (FITC) signals are from the chromosome 2 centromere probe pB54D in each case.

Table 1 Tiling path probes and FISH results for families 1 and 2

Band	Probe name	Mb (from telomere)	Family 1	Family 2
2q13	RP11-368A17	113.1	Not tested	Normal
2q13	RP11-339F22	113.4	Not tested	Normal
2q13	RP11-65I12	113.6	Not tested	Normal
2q13	RP11-480C16	113.8	Not tested	Normal
2q13/q14.1	RP11-395L14*	114.0	Not tested	Normal
2q14.1	RP11-432G15	114.1	Not tested	Deleted
2q14.1	RP11-295D2	114.3	Not tested	Deleted
2q14.1	RP11-141B14	114.3	Not tested	Deleted
2q14.1	RP11-100M8	115.5	Normal	Deleted
2q14.1	RP11-157G6	116.1	Normal	Deleted
2q14.1	RP11-252I18	116.3	Normal	Deleted
2q14.1	RP11-59P4	117.2	Deleted	Deleted
2q14.1	RP11-98C1	118.4	Deleted	Normal
2q14.2	RP11-19E11	119.3	Deleted	Normal
2q14.2	RP11-77A13	119.4	Normal	Not tested
2q14.2	RP11-17N4	119.5	Normal	Not tested
2q14.2	RP11-393J17	119.7	Normal	Not tested

Notes: Mb from telomere indicates distance to the telomeric end of each BAC.

Bold type indicates G-dark band and deleted BACs.

*BAC containing the 2qFus ancestral fusion site.⁸

Family 2 del(2)(q13q14.1)

This deletion of 2q13 to 2q14.1 was originally ascertained in a female of 38 after three miscarriages. She had two children already and her pregnancy at the time was chromosomally normal. The same deletion was found in her mother who had two children and no history of miscarriages.⁷ YACs 786a12 and 817b4 were deleted, 791f4 partially deleted and the deletion estimated at 4.5–6.0 Mb. In order to test the hypothesis that this deletion was the same as that in family 1, we remapped the deletion and found seven clones were deleted (Table 1). The proximal breakpoint was mapped to the 22 kb region of overlap between BACs 395L14 and 432G15 in band 2q14.1 in both mother and daughter. This breakpoint is adjacent to the ancestral great ape chromosome fusion site (2qFus) which is contained within BAC 395L14 close to the overlapping segment of 432G15.⁸ The distal breakpoint was within the 1 Mb between BACs 59P4 and 98C1 within the same band. The karyotype was, therefore, revised to 46,XX,del(2)(q13q14.1).ish del(2)(q14.1q14.1)(395L14+,432G15-,59P4-,98C1+). The minimum size of the deletion was 3.21 Mb and the maximum size 4.27 Mb. The minimum interval contains four known and eight novel genes (Table 2).

The minimum combined length of the deletions in families 1 and 2 was ~5.25 Mb containing 32 genes of which nine were known and 23 novel (Table 2). The maximum size of the overlap between the two deletions was a gene poor region of ~1.9 Mb.

Discussion

The combined results from the overlapping 2q deletions in families 1 and 2 imply that 5.25 Mb from 2q13 to 2q14.2 including 32 genes can be deleted without phenotypic consequences. However, in family 1, the same 2–3 Mb deletion was present in the affected proband and his phenotypically normal father and sibling. There are at least three possible explanations for this apparent discrepancy:

1. The deletion is coincidental to the phenotype of the proband (and possibly his sister) and the suggested diagnosis of PEHO results from an independent recessive condition in this consanguineous family.
2. The deletion may have unmasked a recessive allele at one of the 20 genes within the deleted segment despite the fact that it represents only ~0.1% of the genome.
3. A single copy of the homeobox engrailed 1 (*EN1*) transcription factor (OMIM 131290) gene may be more susceptible to variations in the timing or level of its expression or to variable dosage compensation by its *EN2* paralog in 7q36 (OMIM 131310).

EN1 is an attractive candidate gene as En1 behaves in a dosage sensitive manner in the mouse, is directly involved in cerebellar phenotypes⁹ (the cerebellum was slightly smaller in the proband), has a role in the direction of foliation⁹ (gyral formation was simplified in the proband) and combines with En2 in the formation of the optic tectum¹⁰ (the proband had hypoplastic optic discs). Evidence both for¹¹ and against¹² an association between autistic spectrum disorder and SNPs at the paralogous *EN2* gene has also been presented but it is not clear that upregulation of *EN2* could account for the developmental delay found in the proband.

Nevertheless, family 1 indicates that heterozygous deletions that include *EN1* are compatible with an apparently normal phenotype in man. Several of the other known genes within the deleted regions in families 1 and 2 also have related genes elsewhere (Table 2). It is therefore possible that hemizyosity for these genes is more easily tolerated as suggested for haplosufficient deletions of 2p12.¹³ In this context, it is interesting that replacement of En1 with En2 sequences rescues the En-1 mutant phenotype in the mouse¹⁴ and that dosage compensation of En1 by En2 has already been invoked to explain the suppression of the En-1 cerebellar phenotype by a change of genetic background.⁹ Human *EN1* (NM_001426) and *EN2* (NM_001427) are 82% homologous at the mRNA level and, although expression of *EN2* is more restricted than that of *EN1*, upregulation of paralogous loci has been recorded in single gene knockouts in yeast, even when the paralogs are not coexpressed.¹⁵ The insulin induced protein 2 (*INSIG2*) gene (NM_016133) is also between 75 and 83% homologous with the three transcript variants

Table 2 Known hemizygous genes in families 1 and 2

Gene name	Band	Start – Finish (bp)	Gene description	Expression (ungene)	Gene/protein family	Function	MIM/OMIM number	Other gene/protein family locations
Family 2								
1. <i>SLC35F5</i>	q14.1	114188161– 114230630	Solute carrier family 35, member F5	Wide; all stages (Hs.292509)	Ambiguous	—	—	None
2. <i>ACTR3</i>	q14.1	114363767– 114432395	Actin-like protein 3	Ubiquitous; all stages (Hs.433512)	Actin 3 related 3	Actin polymerization	604222	7 genes in all which form the ARP2/3 complex
3. <i>XM_372953.2</i>	q14.1	114733853– 114734349	Similar to Selenide, water dikinase 1	—	—	—	—	None
4. <i>DPP10</i>	q14.1	114916586– 116316706	Inactive dipeptidyl peptidase 10	Wide; all stages (Hs.176247)	Dipeptidyl peptidase	No known dipeptidyl peptidase activity	608209	3 other gene members
Family 1								
5. <i>DDX18</i>	q14.1	118288485– 118306183	ATP-dependent RNA helicase (DEAD-box protein 18)	Ubiquitous; all stages (Hs.363492)	DEAD box protein 18	Putative ATP-dependent RNA helicase	606355	10 other Ensembl genes
6. <i>Q9BSF2</i>	q14.1	118390396– 118390500	No description	—	Unknown	—	—	None
7. <i>NP_06917.2</i>	q14.1	118393402– 118487939	No description	—	Ambiguous	—	—	1 other Ensembl gene
8. <i>INSIG2</i>	q14.1	118562280– 118583826	Insulin-induced protein 2	Wide; all stages (Hs.7089)	Insulin-induced protein	Control of sterol regulatory elements	608660	<i>INSIG1</i> in 7q36
9. <i>EN1</i>	q14.2	119316003– 119321989	Homeobox protein engrailed-1	Wide; all stages (Hs.271977)	Engrailed Homeobox	Pattern formation during CNS development	131290	<i>EN2</i> in 7q36

Note: G-dark bands in bold.

of the *INSIG1* paralog (NM_198337, 198336 and 005542) which maps with *EN2* to 7q36.

The proband in family 2 had been ascertained for miscarriages,⁷ but it is difficult to see how these could have been caused by a deletion that is compatible with a normal phenotype, especially when the carrier mother had no miscarriages herself and most simple deletions are transmitted without causing additional imbalances.⁶

These deletions are immediately adjacent to the site at which the ancestral great ape chromosomes 2A and 2B fused to produce human chromosome 2.⁸ Thus, the terminal orthologous 5.25 Mb of the short arm of the ancestral chromosome 2B (PTR 13 in chimpanzee) can apparently be deleted in humans and gain or loss of 32 subtelomeric genes does not necessarily have detectable phenotypic consequences.¹⁶ The 2q fusion region is believed to be inherently unstable¹⁷ but, without precise identification of the breakpoint, we can only speculate that this might have contributed to the formation of the proximal breakpoint in family 2.

In conclusion, family 1 illustrates the difficulties in making a clear diagnosis even when the gene content of a particular imbalance is known. Nevertheless, families 1 and 2 provide further evidence that deletions of regions of low gene density are compatible with a normal phenotype.¹³ These results will be added to the Chromosome Anomaly Collection of transmitted imbalances for future reference (www.ngsl.org.uk/Wessex/collection/).

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