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IQCJ–SCHIP1, a novel fusion transcript encoding a calmodulin-binding IQ motif protein

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Abstract

The existence of transcripts that span two adjacent, independent genes is considered rare in the human genome. This study characterizes a novel human fusion gene named *IQCJ–SCHIP1*. *IQCJ–SCHIP1* is the longest isoform of a complex transcriptional unit that bridges two separate genes that encode distinct proteins, IQCJ, a novel IQ motif containing protein and SCHIP1, a schwannomin interacting protein that has been previously shown to interact with the Neurofibromatosis type 2 (NF2) protein. *IQCJ–SCHIP1* is located on the chromosome 3q25 and comprises a 1692-bp transcript encompassing 11 exons spanning 828 kb of the genomic DNA. We show that *IQCJ–SCHIP1* mRNA is highly expressed in the brain. Protein encoded by the *IQCJ–SCHIP1* gene was localized to cytoplasm and actin-rich regions and in differentiated PC12 cells was also seen in neurite extensions.

Keywords: Paracentric inversion; Schwannomin interacting protein; Neurofibromatosis type 2; Language disorder; Autism; Calmodulin

In 1940s, Beadle and Tatum proposed the ''one gene, one protein hypothesis'', which holds that a single gene codes for the production of one protein. We now know that one gene codes one polypeptide and that proteins can be made of more than one polypeptide chain. In prokaryotes, genes are assembled into operons, a cluster of closely spaced gene transcribed from a single regulatory region at the 5′ end of the cluster [1]. Operons encode multiple structurally or functionally related genes from a single transcript that translates into separate independent proteins [2]. In eukaryotes on the other hand, each gene is transcribed separately with independent transcriptional controls for each gene. Occasionally, two adjacent independent genes in the same orientation can merge into a single contiguous RNA transcript called a fusion or chimeric gene but in contrast to polycistronic operons it encodes one distinct fusion protein [3–6]. Gene fusion event might be an additional mechanism to create protein diversity by changing the function of connected proteins and might be advantageous to the organism. Although fusion gene transcripts are considered rare in the human genome [3,4] isolation and characterization of such transcripts can allow for the identification of new functionally significant proteins.

In this study, we identified a novel human fusion transcript located on chromosome 3q25, called *IQCJ–SCHIP1*. We show that *IQCJ–SCHIP1* bridges two adjacent genes that encode distinct proteins, IQCJ, a novel IQ motif containing protein and SCHIP1, a schwannomin interacting protein. We also show that IQCJ–SCHIP1 protein contains a unique calmodulin-binding IQ motif at the N-terminus not shared with its shorter isoform SCHIP1, suggesting a distinctive function for this protein. SCHIP1 has been previously characterized and was shown to be associated with neurofibromatosis type 2 (NF2) protein, a tumor suppressor protein, mutation in which predispose to the

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development of NF2 disorder [7,8]. *IQCJ–SCHIP1* gene was found to be located in the close proximity to the inversion breakpoint in a 6-year-old girl with significant impairment in both expressive and receptive language abilities carrying an inversion of chromosome 3q25–29 inherited from her father, who was also language delayed. The gene was not directly interrupted by the 3q25 inversion breakpoint but its expression in lymphoblastoid cells was significantly reduced, presumably via a position effect. Clinical features of the patient and characterization of the 3q29 inversion breakpoint were described in our previous study [9]. Here, we describe the characterization of *IQCJ–SCHIP1* as a novel human fusion gene and discuss its putative biological function.

Materials and methods

Fluorescent in situ hybridization (FISH). FISH experiments were performed according to standard procedures [10]. Metaphase chromosome spreads were prepared from lymphoblast cell lines established from the patient and the father. Bacterial artificial chromosome (BAC) DNA probes spanning the 3q25–q29 region were chosen using the UCSC genome browser (http://genome.ucsc.edu/). BAC DNA was isolated according to standard protocols (Qiagen) and end-sequenced to confirm position on the reference sequence. One microgram of DNA was labeled with the Biotin-Nick translation mix™ or the DIG-Nick translation mix™ (Boehringer Mannheim) as described by the supplier. Spreads were then observed on an epifluorescent microscope (Zeiss). Long-range PCR (Sv1D and Sv1P) probes, approximately 7.2 kb in size, were amplified from BAC genomic DNA and used to narrow the breakpoint (see Fig. 1). Sv1D and Sv1P were the only two probes not tested in the girl due to a limited availability of biological sample. Primers for the Sv1P product were chosen within *IQCJ–SCHIP1* (AK096479/DQ157848) intron 1 (forward 5′-GGGACAAAAGGAGGTGTCAA-3′ and reverse 5′-CAACTCCC CAGAGCAAGAAG-3′) and upstream from the ATG of *IQCJ–SCHIP1* (forward: 5′-GAAAGAATCCCTGGTGCAAA-3′ and reverse 5′-CAC GTGGACATCACCTTGTC-3′).

Human tissue expression. Various human adult and fetal cDNA tissues (Clontech) were used for semi-quantitative PCR analysis. To determine tissue specificity of the *IQCJ–SCHIP1* transcript we used primers between exon 1a and exon 2: forward 5′-CGATCCAGTCTCCTTTCACC-3′ and reverse 5′-AGATCAGCGACGGGAGACT-3′ (see Fig. 3). Amplification of 3′UTR, shared by all *SCHIP1* isoforms, was used as a control with the following primers: forward 5′-GCCATTCAACCAGAGAACAAG-3′, reverse 5′-GGAACCCTCTGAATCCAACA-3′.

RNA isolation and SYBR Green real-time PCR. Total lymphoblast RNA (2 μg RNA) from the father and normal controls was extracted with the RNeasy Mini Kit (Qiagen), treated with RNase-free DNase (Qiagen) according to the manufacturer's instructions, and reverse-transcribed into cDNA with Superscript reverse transcriptase (Invitrogen) using oligo(dT) primers, followed by PCR using gene-specific primers. The expression level of *IQCJ–SCHIP* was determined in the father's lymphoblasts by SYBR Green real-time PCR (Applied Biosystems). Aspects of real-time quantitative RT-PCR using the ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems) have been described elsewhere [11]. Quantitative values were obtained from the threshold cycle (C_t) number at which the increase in the signal associated with exponential growth of PCR products begins to be detected using PE Biosystems analysis software, according to the manufacturer's manuals. The amount of *IQCJ–SCHIP* transcripts was normalized to the ubiquitously expressed *GAPDH* housekeeping gene. PCR amplifications were always performed in triplicate. Primers used for *IQCJ–SCHIP1* were chosen within exons 1b and 1c (see Fig. 1b). Forward primer: 5′-GAACTGAAAAGATTGCAGA ATCC-3′ and reverse primer: 5′-TTTGATTCCAAGGGCTGTAGA-3′. Primers for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were: forward 5′-CCTCAACGACCACTTTGTCA-3′ and reverse 5′-CCCCT CTTCAAGGGGTCTAC-3′. Primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Parallel PCRs were performed with *IQCJ–SCHIP1*- and *GAPDH*-specific primers on 1 μl cDNA product from the same reverse transcription reaction of the father or control (from 12 healthy individuals). SYBR Green real-time PCR was also used to determine the expression level of *IQCJ–SCHIP* in fetal and adult brain.

PCRs were performed using an ABI Prism 7700 sequence detection system and the SYBR Green PCR core reagent kit (Perkin-Elmer Applied Biosystems). The thermal cycling conditions entailed an initial denaturation step at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s, and 65 °C for 1 min. Results were presented as ratio of *IQCJ–SCHIP1* to *GAPDH*.

Northern blot analysis. Multiple-tissue Northern blots containing human or mouse poly(A)$^+$ RNA (2 μg per lane) were used (Clonetech).

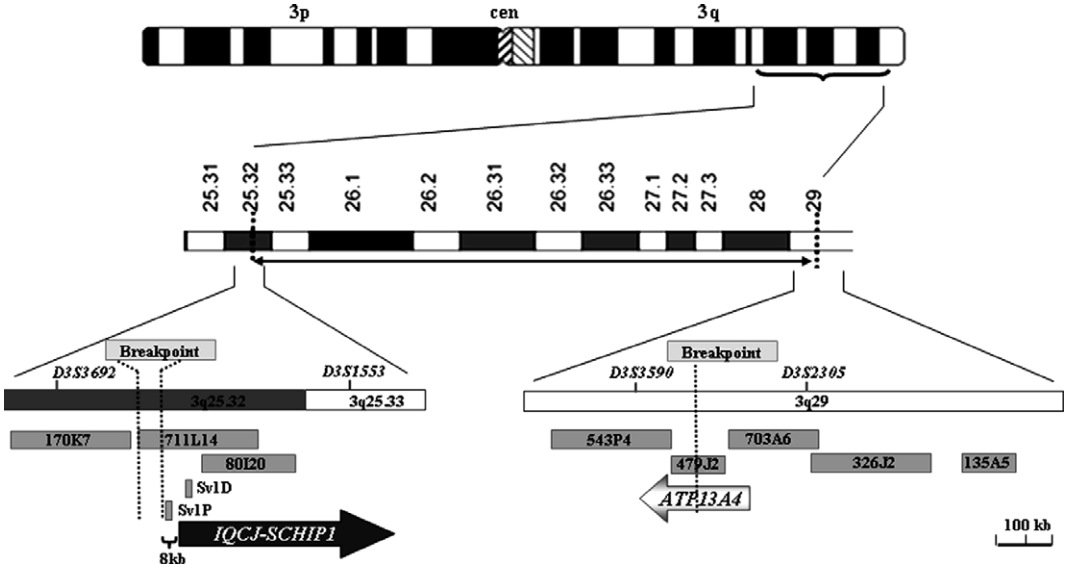

Fig. 1. Mapping of the 3q25 inversion breakpoint. Schematic diagram showing location of the breakpoint on chromosome 3 and selected BAC clones and long-range PCR (Sv1P and Sv1D) used as probes.