Outlook

Arles, 4 January 1889.

'When I come out I shall be able to take my little road here again, and soon the fine weather will be coming and I shall begin again on the orchards in bloom.'

(Vincent to his brother Theo while recovering in the hospital from his self-mutilation)

'Self-Portrait with Grey Felt Hat' – France, 1887, Vincent van Gogh.

Vincent van Gogh (1853 – 1890) became a pioneer of what came to be known as Expressionism and has had an enormous influence on 20th century art. His paintings and drawings include some of the world's best known, most popular and most expensive pieces. His brief, turbulent, and tragic life is thought to epitomise the mad genius legend. Van Gogh's ailment was characterised by episodes of acute mental derangement and disability which were separated by intervals of lucidity and creativity. The underlying condition leading to his mental illness is uncertain. Among others, bipolar affective disorder, schizophrenia, neurosyphilis, epilepsy, alcoholism and lead poisoning all have been proposed as possible causes [Arnold, W.N. (2004). *J Hist Neurosci* 13, 22-43].

The long-term aim of our research is to understand the mechanisms underlying human cognition, brain function and brain development. Eventually, this knowledge may aid in the design of targeted therapeutic interventions, enhanced diagnostic approaches and improved genetic counselling, all of which will relieve the burden on mentally impaired individuals and their families.

Identification of MR genes is a powerful starting point towards achieving these aims. However, the genetic heterogeneity and clinical variability of mental disability considerably hamper a global understanding of human brain function. After having established *hKIAA1202* as a candidate MR gene, we will therefore try to situate it in a broader context. Uncovering biochemical, cell biological and genetic hKIAA1202 interactions will expand the knowledge of molecular pathways and cellular mechanisms underlying human brain function.

A. Further confirmation of *hKIAA1202* as a gene involved in cognition

Functional assays comparing WT and patient-specific variants of *hKIAA1202* will differentiate between mutations and polymorphisms.

While absent from large control panels, one silent and two missense exchanges were recovered in mentally retarded patients. However, these sequence variants could still be rare polymorphisms independent from the MR observed in the patients. Functional analyses are needed to identify the mutations among these exchanges.

Perhaps the silent p.E474E (c.1422A>G) exchange affects an ESE, leading to aberrant splicing[721]. This possibility could be investigated by employing a splicing assay in which WT and c.1422A>G variants of exon 4 are compared. The c.1422A>G substitution could be created using *in vitro* mutagenesis. Both variants should then be cloned into a so-called minigene vector consisting of two splicing-competent exons separated by an intron which contains the MCS[1168]. Transfection in a mammalian cell line, followed by RT-PCR could verify if abnormal splicing indeed occurs. Employing vector-specific primers will avoid amplification of endogenous *hKIAA1202*. Since alternative splicing of exon 4 has not been observed, primers specific to *hKIAA1202* exons 3 and 5 could be used to test the situation in cell lines

from the affected family members carrying the c.1422A>G transition. Aberrant splicing could be studied at the protein level employing the α-hKIAA1202 antibody.

Since the first missense exchange we recovered (c.4116G>T, p.L1372F) is situated in the ASD2 domain, the ASD2 assay from Dietz *et al.* could be employed[1143]. In this test, a fusion construct between the N-terminal portion of ShrmS[777] and the hKIAA1202 ASD2 domain is over-expressed in MDCK cells, leading to apical constriction while leaving the basal surface unchanged[1143]. Comparison of WT and p.L1372F ASD2 domains in such an assay should indicate whether or not the ability of the latter to induce constriction is altered.

Further comparison of the Stocco dos Santos variant (p.S1089L), the second missense alteration we identified, with WT hKIAA1202 is warranted. The subcellular localisation of the over-expressed variant was indistinguishable from that of the WT[199]. In light of the fact that p.S1089L is situated near the EVH1-BS, which has been shown to be important in Actin remodelling pathways[776], a possible impairment of the Stocco dos Santos variant in the ability to translocate F-actin to ectopic sites in the cell, as has been shown for MOM-tagged WT hKIAA1202, is an obvious starting point for further investigations.

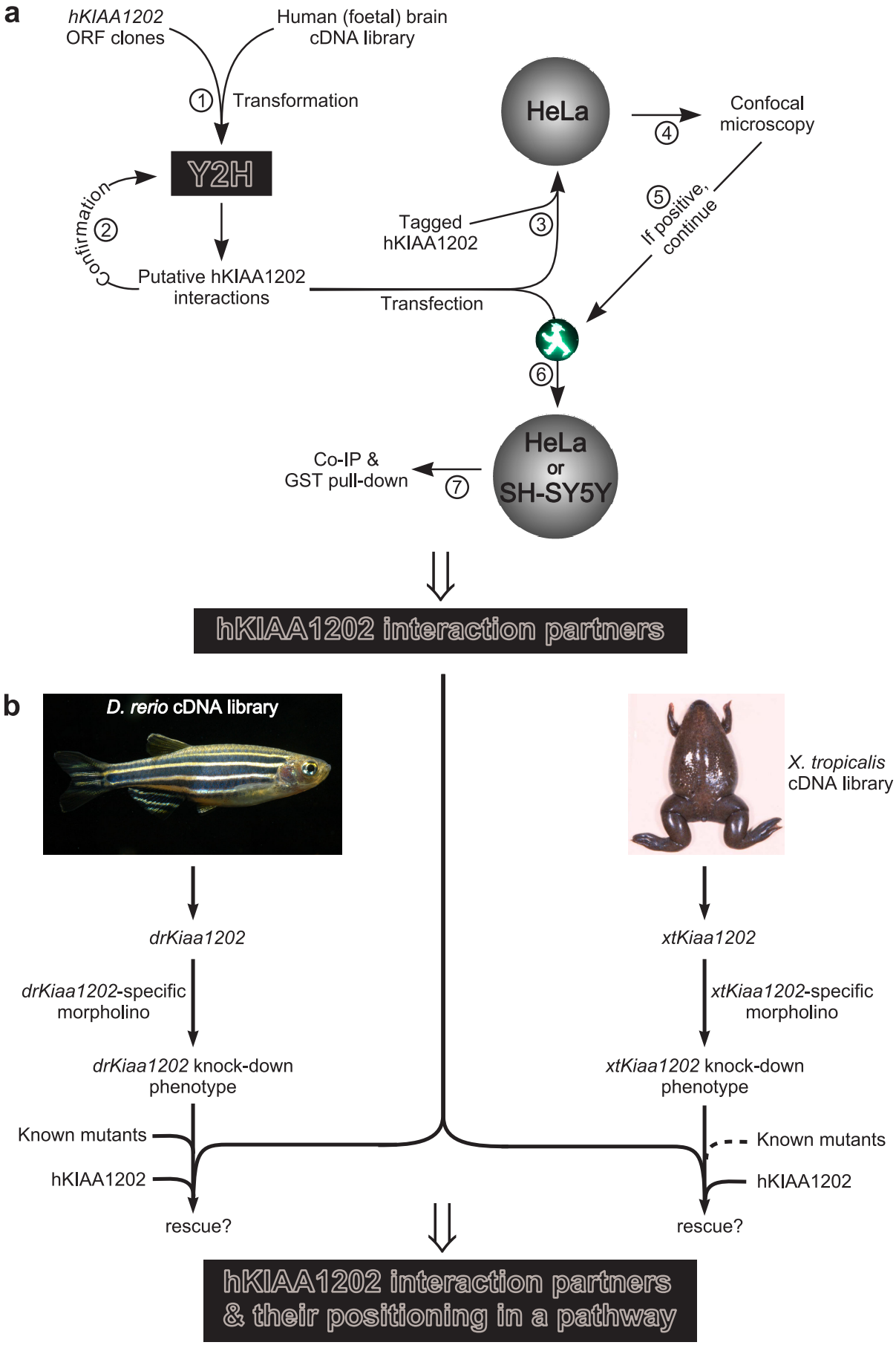
B. Biochemical interactions

Putative interactions, including that with F-actin, and specific isoforms need further study in order to understand hKIAA1202's biology at the cellular level.

Putative hKIAA1202 interaction partners resulting from Y2H screening need to be confirmed in mammalian cells; including those obtained with parts of the ORF that were not covered in the initial screen. In order to interact, proteins must localise to the same subcellular site, so co-localisation studies using confocal microscopy represent a straightforward initial selection criterion. Subsequent co-IP and GST pull-down experiments could then be employed to verify the interactions of these pre-selected candidates with hKIAA1202 (Fig. V-1a). In addition, co-localisation studies, preferably in primary neurons, of endogenous hKIAA1202 with neuronal markers labelling specific subcellular domains, such as PSD-95 to

Fig. V-1 | *Next page.* **Future studies on hKIAA1202 will involve the identification of interaction partners and placing them in a pathway.**

a. Flowchart to identify uncharacterised biochemical and molecular hKIAA1202 interactions.
b. Diagram to identify possible cell biological functions for hKIAA1202 through study of its *D. rerio* and *X. tropicalis* homologues. This approach also allows placing Kiaa1202 and its putative interaction partners, recovered as outlined in panel a, in a pathway.


a
hKIAA1202 ORF clones
Human (foetal) brain cDNA library
1 Transformation
Y2H
2 Confirmation
Putative hKIAA1202 interactions
HeLa
4 Confocal microscopy
5 If positive, continue
Tagged hKIAA1202
3
Transfection
6
HeLa or SH-SY5Y
Co-IP & GST pull-down
7
hKIAA1202 interaction partners
b
D. rerio cDNA library
X. tropicalis cDNA library
drKiaa1202
xtKiaa1202
drKiaa1202-specific morpholino
xtKiaa1202-specific morpholino
drKiaa1202 knock-down phenotype
xtKiaa1202 knock-down phenotype
Known mutants
hKIAA1202
rescue?
Known mutants
hKIAA1202
rescue?
hKIAA1202 interaction partners & their positioning in a pathway

stain the post-synaptic density[775,1169], are necessary to elucidate the nature of the discontinuous hKIAA1202 signal observed in differentiated neuronal cell lines.

The nature of the hKIAA1202 – F-actin interaction deserves further delineation. Time-lapse recordings and longitudinal tracking of virally delivered EGFP-tagged hKIAA1202 in primary hippocampal neurons may shed light on its migration during differentiation. Simultaneous tracing of labelled Actin will allow *in vivo* visualisation of the hKIAA1202 – F-actin interaction.

One issue that certainly requires disentangling is the function and regulation of the different *hKIAA1202* isoforms, as particular non-characterised transcripts appear to be unaffected in the translocation patients. Screening of cDNA libraries seems a useful start in collecting a comprehensive set of isoforms. While isoform-specific multiple tissue northern hybridisations may reveal tissue specificity of certain transcripts, over-expression of each isoform may uncover distinct expression patterns at the subcellular level. α-hKIAA1202 western hybridisations of fractionated cell lysates could be employed to verify the results of the latter series of experiments. Although elaborate, in the end it may be worth raising isoform-specific antibodies.

The knowledge gained from these investigations would likely be important in understanding the biology of hKIAA1202 at the cellular level.

C. Cell biological & genetic interactions

Rescuing knock-down of *Kiaa1202* in *D. rerio* and *X. tropicalis* with possible interactors would place them in a pathway, which may then be extended by setting up genetic crosses.

The zebrafish *D. rerio* and the frog *X. tropicalis* are excellent model organisms to study cell biology, vertebrate development and developmental genetics. The advantages of *D. rerio* include its transparent embryos, its amenability to microinjection and the existence of a large number of mutants that are readily detected using morphological criteria[1170,1171]. The major drawback is its genetic polyploidy, which may result in redundancy problems. *X. tropicalis* embryos are also amenable to microinjection, and in contrast to its better known cousin *X. laevis*, *X. tropicalis* is diploid and has a fast generation time. The major disadvantage is its relatively recent appearance as an experimental system, which is reflected, for example, in the limited number of characterised mutants.

Preliminary RT-PCR results indicated that *drKiaa1202* is not expressed during the first four days of embryogenesis. However, it should be noted that *Kiaa1202* expression is generally very low and that *drKiaa1202* could be expressed in specialised cells, such as is the case for *X. laevis xShroom 3*, a member of the Shroom family that is primarily expressed in a small subset of cells undergoing apical constriction[1144]. Therefore, *drKiaa1202* may be expressed during embryogenesis at levels below the threshold of detection. Expression of *xShroom 3* and *xApx*, another member of the Shroom family, during embryogenesis[1144] or in oocytes[779], respectively suggests that *xtKiaa1202* may also be expressed during embryogenesis.

After identification of *drKiaa1202* and *xtKiaa1202*, these assumptions can readily be tested using morpholino technology to knock down *Kiaa1202* expression and allow screening for morphological changes[1172]. Comparison of the *drKiaa1202* knock-down phenotype with that of characterised mutants would point out putative members of a Kiaa1202 pathway or provide possible links to known pathways. Rescue experiments of *drKiaa1202* and *xtKiaa1202* knock-downs could be performed with (i) hKIAA1202 to settle the degree of functional homology, (ii) *D. rerio* and/or *X. tropicalis* homologues of earlier identified hKIAA1202 interaction partners to position them in a pathway with respect to Kiaa1202 and (iii) candidate zebrafish (and frog) proteins suggested by the phenotypic comparison with other mutants to identify novel Kiaa1202 interactions. Depending on the character of the interaction, the latter two series of rescue experiments may or may not be pursued in parallel in zebrafish and frog (Fig. V-1b).

Finally, it would be interesting to feed the insights gained from these studies into meaningful *D. rerio* and *X. tropicalis* crossing schemes so that wide-range genetic interactions affecting the Kiaa1202 pathway could be established.