Severe insulin resistance and intrauterine growth deficiency associated with
haploinsufficiency for INSR and CHN2: new insights into synergistic
pathways involved in growth and metabolism

Running Title: Insulin resistance and short stature

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Objective: Digenic causes of human disease are rarely reported. Insulin via its receptor, encoded by INSR, plays a key role in both metabolic and growth signaling pathways. Heterozygous INSR mutations are the most common cause of monogenic insulin resistance. However, growth retardation is only reported with homozygous or compound heterozygous mutations. We describe a novel translocation [t(7;19)(p15.2;p13.2)] co-segregating with insulin resistance and pre- and post-natal growth deficiency. Chromosome translocations present a unique opportunity to identify modifying loci therefore our objective was to determine the mutational mechanism resulting in this complex phenotype.

Research design and Methods: Breakpoint mapping was performed by Fluorescence in-situ hybridisation (FISH) on patient chromosomes. Sequencing and gene expression studies of disrupted and adjacent genes were performed on patient derived tissues.

Results: Affected individuals had increased insulin, c-peptide, insulin/c-peptide ratio and adiponectin levels consistent with an insulin receptoropathy. FISH mapping established that the translocation breakpoints disrupt INSR on chromosome 19p15.2 and CHN2 on chromosome 7p13.2. Sequencing demonstrated INSR haploinsufficiency accounting for elevated insulin levels and dysglycaemia. CHN2 encoding beta-2 chimerin was shown to be expressed in insulin sensitive tissues and its disruption to result in decreased gene expression in patient derived adipose tissue.

Conclusions: We present a likely digenic cause of insulin resistance and growth deficiency resulting from the combined heterozygous disruption of INSR and CHN2; implicating CHN2 for the first time as a key element of proximal insulin signaling in-vivo.
The genetic susceptibility to insulin resistance can involve the disruption of a single gene (e.g. *INSR*) or may involve the interplay of many genetic loci (including *PPARG*, *FTO*, *HNF1B* etc). However, there is currently only one known digenic disorder of insulin resistance, resulting from mutations in *PPARG* (peroxisome proliferator activated receptor gamma) and *PPPIR3A* (protein phosphatase 1 regulatory subunit 3) (1). Compound heterozygous mutations in these genes which are primarily involved in carbohydrate or lipid metabolism respectively can combine to produce a phenotype of extreme insulin resistance and lipodystrophy (1). Interestingly, individuals who possess only one mutation have normal insulin levels demonstrating that disruption of both genes and therefore pathways are necessary to result in disease (1).

*INSR* encodes the insulin receptor with a key role in both major arms of the insulin signaling pathways, specifically, the metabolic pathway mainly via IRS-1/Akt2/AS160 signaling and the growth pathway mainly via IRS-2/ERK signaling (2). *INSR* mutations are the commonest cause of monogenic insulin resistance and cause a clinical spectrum of disease ranging from Type A insulin resistance to the most severe form of insulin receptoropathy Leprechaunism (also known as Donohue syndrome) (3-6).

Growth is a complex biological process with multiple interacting pathways. The key pathways involved in growth include the insulin signaling pathway and the growth hormone/insulin-like growth factor (IGF) pathway (7-9). Intra-uterine growth is also regulated by multiple foetal and maternal factors including genetic and epigenetic factors and various environmental factors (10).

We describe a family with a reciprocal translocation [(t(7;19)(p15.2;p13.2)) co-segregating with insulin resistance and pre- and post-natal growth deficiency. We demonstrate that the breakpoint on chromosome 19 disrupts *INSR* causing monoallelic expression. Haploinsufficient *INSR* individuals have Type A insulin resistance with no apparent severe growth deficiency (5). Given the short stature and intra-uterine growth retardation (IUGR) also seen in this family, we hypothesised this could either be due to complete loss of the functional *INSR* protein (due to the second *INSR* allele harbouring a mutation or due to a dominant negative effect of a mutant *INSR* protein), or a digenic syndrome due to disruption of a second gene involved in growth by the chromosome 7 breakpoint. The first two hypotheses were excluded by the demonstration of a normal *INSR* DNA sequence and monoallelic *INSR* expression, whilst further cytogenetic analysis established that the second breakpoint on chromosome 7p15.2 disrupted *CHN2* encoding beta-2 chimerin. The demonstration that disruption of *CHN2* affects both pre and post-natal growth suggests that chimerins may play an important role in early growth and development.

**RESEARCH DESIGN AND METHODS**

**Subjects:** We report a family with pre- and post-natal growth deficiency, insulin resistance and early onset diabetes (Table 1). The female proband was born at term weighing 1.85 Kg (<5th centile), all other intra-uterine growth parameters were <5th centile (Table 1). Her growth was maintained below the 5th centile. She was hypoglycaemic at birth and treated with intravenous 10% glucose infusion for 36 hours. She has mild dysmorphic features with a triangular face, irregular teeth and hypertrichosis and a masculine appearance and no history of
miscarriage. A diagnosis of diabetes requiring insulin treatment was made at age 15 years since then the proband has been on insulin therapy with variable dosage notably, requiring less insulin throughout pregnancy and dietary treatment alone for 2 months post-natally and she is currently managed with gradually increasing insulin doses (Online Supplementary Methods Table 1). Fasting insulin and c-peptide levels are greatly elevated (Table 1). Karyotype analysis identified the following karyotype: [46, XX, t(7;19)(p15.2;p13.2)].

The proband’s son was the first born child of a non-consanguineous marriage delivered by caesarean section at 35 weeks gestation. Aminocentesis at 16 weeks gestation identified the following karyotype [46. XY, t(7;19)(p15.2;p13.2)]. He weighed 1.9Kg (<10th centile) and his length was <5th centile (Table 1). He has no dysmorphic features. He had recurrent neonatal hypoglycaemia and was noted to have pre-prandial hypoglycaemia and post-prandial hyperglycaemia aged 3 months. Fasting insulin and c-peptide levels were greatly elevated (Table 1). He is currently aged 22 months and continues on dietary management and his growth trajectory remains below the 3rd centile.

The proband’s parents are non-consanguineous and have no evidence of diabetes, growth retardation or dysmorphic features. The height of the proband’s mother, father and older sister is 162cm, 174 cm and 173cm respectively. Chromosomal analysis in both parents revealed a normal karyotype.

Biochemical analysis:- Insulin and c-peptide levels were assayed using a radio-immunoassay (Immunotech, Prague, Czech Republic). Adiponectin was assayed using a radio-immunoassay (Linc, Millipore, UK) normative data was acquired from 27 healthy controls.

Bioinformatic tools:- the University of California Santa Cruz (UCSC) website (http://genome.ucsc.edu/), online Mendelian inheritance in man (OMIM) (www.ncbi.nlm.nih.gov) and genesniffer (www.genesniffer.org) were used to identify and prioritise biological candidate genes within the region of both breakpoints.

Chromosome preparation, DNA isolation & establishment of patient cell lines:- Peripheral blood samples were obtained from the proband and her son, and conventional methods used to prepare metaphase spreads for FISH analysis, extract genomic DNA and establish an EBV transformed lymphoblastoid cell line.

Fluorescence In Situ Hybridization (FISH) analysis:- was performed using standard techniques (see Online Supplementary Methods which is available at http://diabetes.diabetesjournals.org).

Gene dosage investigations: Multiplex Ligation-dependent Probe Amplification (MLPA) and SYBR green analysis were used to quantify gene dosage of INSR and CHN2 respectively (Online Supplementary Methods).

Sequencing: All 22 exons and exon-intron boundaries of INSR were amplified and sequenced on an ABI 3700 genetic analyzer (Applied Biosystems, Warrington, UK). Sequences were compared to the published sequence (NM_000208) using Mutation Surveyor v.3.4 (Softgenetics, Cambridge, UK). The entire cDNA sequence of INSR was amplified in nine overlapping fragments and sequenced as above. Coding sequences with known single nucleotide polymorphisms (SNPs) of CHN2 and adjacent biologically plausible genes (GHRHR, JAZF1, GRB10) were amplified and sequenced in patient genomic DNA. All primer sequences are available in the Online Supplementary Methods.

RNA extraction, retro-transcription and gene expression studies: RNA was extracted and retro-transcribed from patient Epstein-Barr Virus (EBV) transformed
lymphoblastoid cell lines and the proband’s subcutaneous adipose tissue obtained by needle biopsy using standard methods (Online Supplementary Methods). A commercially available RNA library from a standard panel of tissues (Human Total RNA Master Panel II) was purchased from Clontech (Saint-Germain-en-Laye, France). Gene expression analysis was performed by quantitative real-time PCR (qRT-PCR) on an ABI 7900HT analyser using inventoried and designed assays (Applied Biosystems). Data were normalised to the mean of three housekeeping genes (HKGs) (11). Details of assays and HKGs are provided in the Online Supplementary Methods available at http://diabetes.diabetesjournals.org.

RESULTS

Karyotype analysis revealed an apparently balanced translocation [t(7;19)(p15.2;p13.2)] in both the proband and her affected son. The proband’s unaffected parents had normal karyotypes suggesting the translocation arose de novo in the proband. We hypothesised that the breakpoint of this translocation disrupted one or more genes involved in the aetiology of the insulin resistance and growth deficiency in both subjects. Bioinformatics identified a total of 67 genes within the breakpoint regions on chromosomes 19p13.2 and 7p15.2. The most plausible biological candidate was INSR on chromosome 19. Biochemical analysis showed raised insulin, c-peptide, insulin/c-peptide ratio and adiponectin levels in both individuals, suggesting disruption of INSR (Table 1).

FISH analysis of proband chromosomes using BACs and fosmids narrowed down the region of the breakpoint on chromosome 19 to a ~10Kb region entirely within the genomic sequence of INSR (Figures 1a and 2a), predicted to be between exons 13 and 15. To investigate possible cryptic microdeletions within the INSR sequence MLPA analysis was performed in both patients. Taking into account the resolution limits of FISH we decided to perform MLPA analysis on a broader DNA region spanning exons 11-17. A microdeletion between 2.4 - 6.6 Kb in size including exons 15-16 was detected (Figure 2c). Direct sequencing of the entire coding region of INSR in patient genomic DNA excluded an INSR mutation. An informative heterozygous single nucleotide polymorphism (SNP) (c.1650G>A; p. A550A) identified in patient genomic DNA was shown to be monoallelic in patient RNA establishing INSR haploinsufficiency and excluding a potential dominant negative mutational mechanism (Figure 2d). Reduced INSR expression in both patient derived EBV cell line and adipose tissue cDNA was also demonstrated (Figures 2e & 2f).

In order to explain the clinical phenotype observed in this family and determine the mutational mechanism for the growth deficiency we also mapped the translocation breakpoint on chromosome 7. A number of strong biological candidate genes for growth map to the region including JAZF1 (12-14) and GHRHR (15). Disruption of both genes was excluded by FISH analysis (data not shown) whilst gene expression studies on patient EBV-transformed lymphocyte cell line derived cDNA, compared to healthy controls demonstrated that JAZF1 expression was not altered (Online Supplementary Results Figure 1). GHRHR is only expressed in the pituitary so it was not possible to investigate patient gene expression levels. To exclude a GHRH receptor defect the proband underwent a growth hormone releasing hormone (GHRH)-arginine stimulation test which illustrated a normal response (Online Supplementary Results Table 1).

Further mapping on chromosome 7 restricted the breakpoint to 25.3Kb entirely within the genomic sequence of CHN2 (Figures 1b & 2b). Gene dosage studies
established that there was no loss of the coding region of CHN2 (data not shown). Monoallelic CHN2 expression could not be demonstrated as no informative coding SNPs were identified in patient genomic DNA. Gene expression studies established that CHN2 is expressed in human brain and insulin sensitive tissues including liver, adipose tissue (subcutaneous and omental) and muscle (Figure 3a). Expression analysis in patient adipose tissue-derived cDNA compared to healthy controls demonstrated reduced CHN2 expression (Figure 3b).

Given the proximity of the translocation breakpoint to an imprinted region on chromosome implicated in Silver Russell Syndrome (SRS) and the possibility of a positional effect of the translocation on gene expression we excluded involvement of GRB10 - the SRS candidate gene in this region - by establishing that GRB10 gene expression levels were normal in patient adipose tissue (Online Supplementary Results Figure 2) (16). Monoallelic GRB10 expression could not be confirmed due to a lack of informative coding SNPs in the GRB10 coding sequence.

**DISCUSSION**

Digenic causes of human disease are rare in the literature but present an opportunity to model possible gene-gene interactions which may provide insights into common metabolic disorders including T2DM. We report a family with a novel reciprocal translocation [t(7;19)(p15.2;p13.2)] resulting in the first reported case of severe insulin resistance, diabetes and growth deficiency resulting from the synergistic disruption of INSR and CHN2.

It is well established that INSR mutations are the commonest cause of monogenic insulin resistance. Most mutations are missense mutations in the beta subunit which have dominant negative activity; however truncating mutations have a similar effect. Biochemical features supporting the diagnosis of an INSR defect in the family described include: markedly elevated insulin and c-peptide levels as a response to severe insulin resistance and due to reduced insulin clearance (17); a raised insulin/c-peptide ratio (17) and raised adiponectin levels relative to the degree of insulin resistance in these subjects (18). This is confirmed by our genetic investigations which demonstrated disruption of INSR by a reciprocal translocation and mono-allelic INSR expression. However, our genetic studies at the INSR locus do not explain the severe pre- and post-natal growth deficiency observed in both subjects. Variable penetrance of INSR haploinsufficiency many result in phenotypic heterogeneity. Published evidence from parents of children with Donohue syndrome shows that haploinsufficient parents have a mildly deranged or normal metabolic phenotype although some may have marked insulin resistance; however, this is usually in the presence of obesity or other risk factors (5, 19).

The family we describe shares some features with the more severe autosomal recessive insulin receptoropathies (Donohue syndrome and RMS). Both affected individuals have an intermediate phenotype with neonatal hypoglycaemia, severe insulin resistance, hyperglycaemia in childhood and intra-uterine and post-natal growth deficiency however they have no evidence of pre-mature mortality. The proband also has mild dysmorphic features with minimal subcutaneous fat, overdeveloped musculature (masculinisation) and abnormal dentition, all features seen in severe autosomal recessive insulin receptoropathies.

. The genotype-phenotype correlation in patients with INSR mutations is likely to be affected by the mutation site and possible modifier loci (20). Moreover, evidence from mice that are double heterozygous for null
alleles in \textit{INSR} and \textit{IRS-1} (\textit{INSR}+/\textit{IRS-1}+/−) genes display a synergistic effect on insulin resistance with a 5 - 50 fold rise in insulin levels despite the expected ~50\% reduction in the protein levels of \textit{INSR} and \textit{IRS-1} (21) suggesting that gene-gene interactions may play a significant role in severe insulin resistance. In this family we have the rare opportunity to define a possible modifier locus by mapping the second breakpoint of the translocation. Mapping of the breakpoint on chromosome 7 demonstrated disruption of \textit{CHN2} which encodes Beta-2 Chimerin. Chimaerins are ligand activated Rac-specific GTPase activating proteins (GAPs) (22) which are expressed in many human tissues especially brain, pancreas and insulin sensitive tissues. Chimaerins are downstream effectors of tyrosine kinase receptors and have been shown to regulate growth in an inhibitory manner via suppression of Rac and ERK phosphorylation (23). Decreased expression of \textit{CHN2} is associated with high-grade malignant gliomas, duodenal adenocarcinomas and breast tumours (23) suggesting that chimaerins are tumour suppressors however increased expression of \textit{CHN2} is reportedly associated with lymphomas (24). We therefore propose that reduced \textit{CHN2} expression, a gene with a known role in growth pathways, contributes to a novel digenic syndrome of insulin resistance and dysglycaemia due to disruption of \textit{INSR} combined with marked pre- and post-natal growth deficiency due to disruption of \textit{CHN2}.

There is a possible role for phenotypic modification caused by disruption of regulation of other biologically plausible genes close to the chromosome 7p breakpoint as there are several strong biological contenders. Although these genes are a long way from the breakpoint, disruption of the spatial relationship between these genes and unknown regulatory elements, as seen with other translocations, cannot be excluded (25).

\textit{GHRHR} maps to chromosome 7p15.2 and mutations in \textit{GHRHR} are a known cause of Dwarfism of Sindh (15). However, a GHRH test performed on the proband confirmed a normal response thereby excluding a defect in the \textit{GHRH} receptor. \textit{JAZF1} on chromosome 7p15.1-15.2, is a recently described T2DM susceptibility gene with a known role in growth (12-14). However, \textit{JAZF1} gene expression levels are not altered either the proband or her son. \textit{SRS} has also been linked to chromosome 7p and the minimal overlap region has been delineated to chromosome 7p11.2 approximately 25Mb away from the breakpoint (26). This region contains multiple biological candidates with the strongest evidence supporting \textit{GRB10} (16). \textit{GRB10} encodes growth factor receptor binding protein 10 which is also known as insulin receptor binding protein and is maternally imprinted (16). Mice carrying maternally inherited targeted deletions of \textit{Grb10} display a growth phenotype which recapitulates the SRS phenotype described in patients (16). However, our investigations have shown that there are no differences in \textit{GRB10} gene expression levels in the proband and her son compared to normal healthy controls.

\textbf{SUMMARY/CONCLUSION:}

We describe a novel cytogenetic defect resulting in a syndrome of severe insulin resistance, dysglycaemia and pre- and post-natal growth deficiency. Our proof of simultaneous heterozygous disruption of \textit{INSR} and \textit{CHN2} without loss of other candidate growth-related genes in the region suggests this represents a novel digenic disorder, and implicates \textit{CHN2} for the first time in insulin’s metabolic and growth promoting actions \textit{in-vivo}.

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Table 1. Clinical & biochemical characteristics of patients with the novel translocation

|                        | Proband                  | Son                  |
|------------------------|--------------------------|----------------------|
| Current age [years]     | 24                       | 2                    |
| Gestational age [weeks] | 40                       | 35                   |
| Birth weight [kg] (5th-95th centile) | 1.85 (2.69 – 4.03) | 1.9 (1.84 - 3.2) |
| Length at birth [cm] (5th-95th centile) | 43 (43.47 – 52.69) | 39 (44.08 - 49.7) |
| Head circumference at birth [cm] | 33 (10th centile) | 32                   |
| Current weight [kg]     | 38                       | 10.3 (3rd centile)   |
| Current height(mid-parental height) [cm] | 145 (161) | 80 (< 3rd centile)   |
| Current BMI [Kg/m^2] (normal range) | 18 (19 - 25) | 16 (15 – 18) |
| Acanthosis nigricans    | No                       | No                   |
| Hypertrichosis(facial, limbs and trunk) | Yes (aged 11 yrs) | No                   |
| Menarche [years]        | 11                       | -                    |
| Age at diagnosis of hyperglycaemia [years] | 15.9 (symptoms from age 10 yrs) | 0.25 |
| Current treatment       | Insulin                  | diet                 |
| Glucose in the neonatal period [mmol/l] (normal >2.6mmol/l ) | 3.2 | 1.4 |
| Fasting glucose at diagnosis [mmol/L] (normal < 7mmol/l) | 11.2 | 2.3 - 8 (pre-prandially) |
| Ketonuria               | trace                    | negative             |
| Auto antibodies (GAD, IA2A) | negative               | negative             |
| Insulin [pmol/L] (5 - 95th centile) | 3724 (15.2 - 159) | 6025 (18 – 46.8) |
| C-peptide [pmol/L] (normal 160 – 1100 pmol/l) | 2931 | 8081 |
| Insulin/c-peptide ratio ( normal < 0.1) | 1.27 | 0.74 |
| Adiponectin [ng/ml] (control mean +/- 95% CI) | 22.3 | 26.1 |
| Triglycerides [mmol/l]  | 0.62 (0.55 – 1.9)       | 1.0                  |
| Cholesterol [mmol/l]    | 3.9 (3.5 – 5.5)         | 4.3 (3.8 – 4.5)      |
| HDL [mmol/l]            | 1.62 (0.8 – 1.8)        | 1.61 (0.82 – 0.94)   |
| LDL [mmol/l]            | 1.68 (0.8 – 2.2)        | 2.05                 |
| IGF-1 (normal range for age) | 873 ng/ml (90–500)  | 203.7 ng/ml (70-380) |
| Proteinuria (<30 mg/24 hrs) | 353.4 mg/24 hrs (pre-pregnancy) | N/A |

*Insulin resistance and short stature*
Figure Legends

**Figure 1a.** Map of the INSR region on chromosome 19p13.2 illustrating the BACs and FOSMIDs selected for FISH analysis.

BACs (approximately 150-200 Kb in size) and Fosmids (approximately 40 Kb in size) overlapping over the entire genomic sequence of INSR were selected and obtained for FISH analysis. Figure also shows the results of FISH analysis which demonstrated that the minimal breakpoint region is 10.8 Kb all within the genomic sequence of INSR.

**Figure 1b:** Map of the CHN2 region on chromosome 7p15.1 illustrating the BACs and FOSMIDs selected for FISH analysis.

BACs (approximately 150-200 Kb in size) and Fosmids (approximately 40 Kb in size) overlapping over the entire genomic sequence of CHN2 were selected and obtained for FISH analysis. Figure also shows the results of FISH analysis which demonstrated that the minimal breakpoint region is 25.3 Kb all within the genomic sequence of CHN2.

**Figure 2a: Fluorescence in situ hybridization (FISH) analysis showing disruption of the BAC CTD-2560C1 containing the genomic sequence of INSR**

The figure shows that the digoxigenin-labelled BAC CTD-2560C1 (red) spans the breakpoint as there are three signals apparent where it hybridizes to chromosome 19, derivative chromosome 19 and derivative chromosome 7. The two chromosome 7 homologs are identified by a FITC-labelled chromosome-specific paint (green). Chromosomes are counterstained with DAPI (blue).

**Figure 2b: Fluorescence in situ hybridization (FISH) analysis showing disruption of the BAC RP11-980H8 containing the genomic sequence of CHN2**

The figure shows that the digoxigenin-labelled BAC RP11-980H8 (red) spans the breakpoint with three signals apparent where it hybridizes to chromosome 7, derivative chromosome 7 and derivative chromosome 19. The two chromosome 7 homologs are identified by a FITC-labelled chromosome-specific paint (green). Chromosomes are counterstained with DAPI (blue).

**Figure 2c: Identification of a cryptic microdeletion (exons 15-16) within INSR using multiplex ligation-dependent probe amplification (MLPA)**

Graph of normalised gene dosage of INSR exons 11-17 and control HNF1A and HNF4A exons run at the same time. Dosage quotients were calculated from average crossing points of triplicate samples, using the comparative Ct (ΔΔCt) method. A ratio of 1 implies normal gene dosage, and a ratio below 0.75 suggests a deletion. Exons 15 and 16 of INSR are deleted with a ratio of less than 0.75.

**Figure 2d: Demonstration of mono-allelic expression of INSR in a patient derived lymphoblastoid cell line**

The exon 8 silent variant c.1650G>A, p.A550A is heterozygous in genomic DNA but sequencing of patient cDNA reveals mono-allelic expression due to the heterozygous SNP only showing one allele at c.1650G.

**Figure 2e: INSR expression is reduced in patient EBV cell line derived cDNA compared to a healthy control**

INSR gene expression studies in patient EBV cell line derived cDNA showed reduced expression in both the proband and her affected son compared to a healthy control sample.
**Figure 2f:** *INSR* expression is reduced in patient adipose tissue derived cDNA compared to 3 healthy matched control samples

*INSR* gene expression studies in the proband’s subcutaneous adipose tissue derived cDNA showed reduced expression compared to that from three BMI-matched samples.

**Figure 3a:** Expression profile of *CHN2* in a panel of healthy human tissues to identify tissue distribution, shows expression in brain and insulin sensitive tissues

The ratio of *CHN2* expression relative to the mean of three housekeeping genes (*PPIA, GAPDH* and *18s*) was analysed in a panel of human tissues. The results displayed are normalised to the tissue with the highest expression (small intestine).

**Figure 3b:** *CHN2* expression is reduced in patient adipose tissue derived cDNA compared to three matched healthy control samples

Gene expression studies to identify the expression levels of *CHN2* (relative to the mean of three housekeeping genes) were performed using a combination of inventoried and designed assays to cover all known transcripts of *CHN2*. All transcripts of *CHN2* were reduced in patient subcutaneous adipose tissue (pt AT) compared to three healthy controls (mean control).
Insulin resistance and short stature

Figure 1b

Gene

BA CS
RP11-57K11 - Distal
RP11-9801F8 - Spans
RP11-963J3 - Proximal

Fosmids
G248P83251P9 - Distal
G248P87873D7 - Spans
G248P88359C10 - proximal

Breakpoint
Chr7:29,465,715-29,491,031

Figure 2a

Figure 2b
Figure 3b (i)

**CHN2 Ex 5-6/HKG**

- Proband: Ratio of CEN2 to mean base-exchange gene expression
- Mean control: Subcutaneous adipose tissue

- Proband: Ratio of CEN2 to mean base-exchange gene expression
- Mean control: Subcutaneous adipose tissue

Figure 3b (ii)

**CHN2 Ex 9-10/HKG**

- Proband: Ratio of CEN2 to mean base-exchange gene expression
- Mean control: Subcutaneous adipose tissue

- Proband: Ratio of CEN2 to mean base-exchange gene expression
- Mean control: Subcutaneous adipose tissue

**CHN2-Exon 12-13/HKG**

- Proband: Ratio of CEN2 to mean base-exchange gene expression
- Mean control: Subcutaneous adipose tissue

- Proband: Ratio of CEN2 to mean base-exchange gene expression
- Mean control: Subcutaneous adipose tissue