



Article

Somatic Genetic Variation in Solid Pseudopapillary Tumor of the Pancreas by Whole Exome Sequencing

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Abstract: Solid pseudopapillary tumor of the pancreas (SPT) is a rare pancreatic disease with a unique clinical manifestation. Although *CTNNB1* gene mutations had been universally reported, genetic variation profiles of SPT are largely unidentified. We conducted whole exome sequencing in nine SPT patients to probe the SPT-specific insertions and deletions (indels) and single nucleotide polymorphisms (SNPs). In total, 54 SNPs and 41 indels of prominent variations were demonstrated through parallel exome sequencing. We detected that *CTNNB1* mutations presented throughout all patients studied (100%), and a higher count of SNPs was particularly detected in patients with older age, larger tumor, and metastatic disease. By aggregating 95 detected variation events and viewing the interconnections among each of the genes with variations, *CTNNB1* was identified as the core portion in the network, which might collaborate with other events such as variations of *USP9X, EP400, HTT, MED12,* and *PKD1* to regulate tumorigenesis. Pathway analysis showed that the events involved in other cancers had the potential to influence the progression of the SNPs count. Our study revealed an insight into the variation of the gene encoding region underlying solid-pseudopapillary neoplasm tumorigenesis. The detection of these variations might partly reflect the potential molecular mechanism.

Keywords: SPT; exome sequencing; genetic variation; SNPs; indels

1. Introduction

Solid pseudopapillary tumor (SPT, also known as solid pseudopapillary neoplasm) is an uncommon but distinct pancreatic tumor with a reported incidence of approximately 2% of all exocrine pancreatic neoplasms [1]. Most SPTs have been diagnosed in females with a mean age of 28 years [2,3], and have always presented characteristics of indolent biological behavior and high rates of long-term survival [1,2]. Surgical resection resulted in better outcome even in metastatic disease [4]. Although multiple studies have allowed insight into SNPs genetic pathogenesis, comprehensive exploration of the variations of the gene coding region has not been performed [4,5].

Variations of *KRAS*, *SMAD4*, *TP53* and *CDKN2A* have never been detected in SPT [5,6], which is different to the molecular changes seen in some malignancies such as pancreatic cancer. However, the

significance of Wnt signaling with β -catenin mutations in SPT has been determined [4]. Almost all patients with SPT have mutations of the somatic β -catenin coding gene (*CTNNB1*), and numerous proteins associated with β -catenin have been detected as dysfunctional [5,7,8]. Normally, neoplasm development has been described as regulated by multiple events instead of a single key protein [9]. In SPT, other gene variations may have a synergistic effect on the biological behavior of the neoplasm.

In the present study, we applied whole exome sequencing to investigate the cause of the genetic variation of solid pseudopapillary tumor. By identifying the prominent variations of indels and SNPs, 95 events were detected which were observed to impact gene function. These events have enabled us to describe the potential molecular pathways involved in the pathogenesis of this disease.

2. Results

We performed whole-exome sequencing of paired SPT tissues from nine patients with SPT confirmed by pathology, including four males (aged from 26 to 51 years) and five females (aged from 25 to 43 years). All patients were diagnosed with pancreatic cystic, solid, or cystic-solid lesions. The clinical features of seven patients with non-metastatic disease and two patients with metastases are listed in Table 1. Each set of paired sequencing data from the neoplasm and adjacent tissues were compared to detect the SPT-specific gene variations.

| Patients | Gender | Age (Years) | Size (mm) | TNM Stage | Location | Distant Metastasis (Yes/No) | CA19-9 Value | Surgical Procedures |
|----------|--------|----------------|--------------|--------------|---------------|--------------------------------|--------------|-------------------------|
| 1 | male | 35 | 18 | Ι | head | No | no abnormal | distal pancreatectomy |
| 2 | male | 33 | 50 | Π | body and tail | No | no abnormal | distal pancreatectomy |
| 3 | male | 26 | 70 | Π | body and tail | No | no abnormal | distal pancreatectomy |
| 5 | female | 43 | 108 | Π | head | Yes | no abnormal | total pancreatectomy |
| 7 | female | 30 | 45 | Π | body and tail | No | no abnormal | distal pancreatectomy |
| 8 | female | 31 | 45 | Π | head | No | no abnormal | pancreaticoduodenectomy |
| 9 | female | 25 | 50 | Π | body and tail | No | no abnormal | distal pancreatectomy |
| 10 | female | 25 | NA | Π | head | No | no abnormal | pancreaticoduodenectomy |
| 11 | male | 51 | 138 | IV | body and tail | Yes | no abnormal | distal pancreatectomy |

Table 1. Clinicopathological characteristics of patients.

2.1. Mononucleotide Variation in Solid Pseudopapillary Tumor of the Pancreas (SPT)

We performed an overview of all the non-synonymous mutations among the coding regions of each of the samples, and 65 prominent single base changes (SNPs) were detected (Table 2, Figure 1). The variations were detected in 56 genes, and *CTNNB1*, a β -catenin protein-coding gene, was found to be mutated in all the patients. In addition, no other general single sequence variation was found (Figure 1A). Almost all of the variations in the alleles were heterozygous mutations, and only one homozygous mutated base in the *MED12* gene had occurred (in patient number 1) (Table 2). Although the sample size investigated was limited, comparison of the incidence of SNPs between each case suggested that more SNPs events occurred in patients with distant metastases (p < 0.01) (Figure 1B). Interestingly, the patients with larger tumor size (diameter >100mm) had more SNPs detected than others with smaller size (Table 1, Figure 1B) (p < 0.01). In addition, the two patients with metastatic disease were older than the others. Moreover, analysis of the SNPs location showed that more mononucleotide variation was distributed in chromosomes 2, 1, and 17 (Figure 1C).

| Table 2. Information of prominent SNPs in each patie | ent. |
|--|------|
|--|------|

| Samples | Gene | Biotype | Transcript | Codon | Chromosome | Alleles |
|------------|----------|----------|---------------------------|---------|------------|---------|
| | C1orf100 | Missense | NM_001012970:p.Tyr78Cys | tAt/tGt | chr01 | het |
| | CTNNB1 | Missense | NM_001098209:p.Asp32Tyr | Gac/Tac | chr03 | het |
| Dationt 01 | MED12 | Missense | NM_005120:p.Arg1295Cys | Cgt/Tgt | chrX | hom |
| Patient_01 | MYO1E | Missense | NM_004998:p.Ser179Arg | agT/agG | chr15 | het |
| | SOS2 | Missense | NM_006939:p.Leu793Ile | Ctt/Att | chr14 | het |
| | UNC13C | Missense | NM_001080534:p.Lys1395Met | aAg/aTg | chr15 | het |

| CTNNBI Missense NM.00108209-p.492City gAr./GC ch01 hei Patient_02 PAPATX Missense NM.0010760-p.409579Aan Gr/GC ch01 hei Patient_03 CTNNBI Missense NM.001276/Cld477As Gr/Atat ch03 hei Patient_03 CTNNBI Missense NM.00109209-p.Gi374Arg Gr/Atat ch03 hei Patient_01 Missense NM.00109209-p.Gi374Arg Gr/Atat ch03 hei CTNNBI Missense NM.00109209-p.Gi374Arg Gr/Atat ch03 hei D/DCK8 Missense NM.2013425, Arg831is Gr/Atat ch13 hei Misr Missense NM.00164217, hig343Arg ch7475 ch14 hei PRU11 Missense NM.00164217, hig343Arg ch7475 ch141 hei PHU11 Missense NM.020157, Lag114Arg Gg7/Cg6 ch171 hei PHU11 Missense NM.020157, Lag114Arg Gg7/Cg6 ch171 hei PHU11 | Samples | Gene | Biotype | Transcript | Codon | Chromosome | Alleles |
|--|-------------|-----------------|----------|-----------------------------|-----------------------------------|------------|---------|
| Patient_02 IE2AFX NEB NEB SIPK Missense Missense NM_0010820Pp.cbg3Apg7 CTp7Cg Cat/At pA/gpC chr02 chr03 het chr02 chr03 Patient_03 CTNNB1 Missense Missense NM_00109820Pp.cliy34Arg CALAT Gga/Aga chr03 het Patient_03 CTNNB1 Missense Missense NM_00109820Pp.cliy34Arg Gga/Aga chr03 het Patient_05 DCK8 Missense NM_00109820Pp.cliy34Arg Gga/Aga chr03 het Patient_05 Missense NM_00109820Pp.cliy34Arg Gga/Aga chr03 het Patient_05 Missense NM_00109820Pp.cliy34Drg8391is cGi/CAt chr01 het PARDI Missense NM_00101977p.1hr921le aC/CAt chr01 het PRD1 Missense NM_001019577p.Gi/HA.grg240C/CAt chr01 het PRD1 Missense NM_001019577p.Gi/HA.grg240C/CAt chr01 het PALU Missense NM_001018572.pls.pls2320 cG/CAt chr01 het PALU Missense NM_0010820Pp.sers37p.ci/HA.grg240C/CAt chr01 het PACTL8 Missense NM_001018472.p.Arg8481is | | CTNNB1 | Missense | NM 001098209:p.Asp32Gly | gAc/gGc | chr03 | het |
| Patient_0.02 NB_B Missense NM_010164507/p.Asg.0797/Asn Git/Asit chr01 het Patient_0.3 CTNNB1 Missense NM_0101096209-p.G1977Asn Gga/Aga chr03 het Patient_0.3 CTNNB1 Missense NM_0101096209-p.G1977Asn Gga/Aga chr03 het Patient_0.3 CTNNB1 Missense NM_001098209-p.Gar77no Git/Att chr03 het DOCK8 Missense NM_00109420-p.Gar77no Git/Att chr01 het VBD1 Missense NM_0010944p.Asg.4280Cys Ggc/Tgc chr01 het PKID11 Missense NM_0010974p.Tp.1fv321e GC/Afc chr04 het PRD1 Missense NM_0102658p.His23Clin GC/AcAc chr08 het PRIDXIN Missense NM_0102658p.Cp.3479Fr GC/AcAc chr08 het PATent_0.07 Missense NM_00109829p.Ser3719Fr GC/AcAc chr08 het PATent_0.07 Missense NM_00109829p.Ser37497 GC/AcAc c | D () () | H2AFX | Missense | NM_002105:p.Leu98Arg | cTg/cGg | chr11 | het |
| $\frac{5H}{Patient_03} \frac{SH}{K} Missense NM_001098209-p.Gly34Arg Gap (Aga dhr0) het Missense NM_002127.p.Ag27H3 Gap (Aga dhr0) het CA(FA) dhr0) het Missense NM_0020127.p.Ag27H3 Gap (Aga dhr0) het CA(FA) dhr0) het CA(FA) Missense NM_001098209-p.Gly34Arg (Gap (Aga dhr0) het CA(FA) dhr0) het Missense NM_001097.p.Nal25Mis GG7(At dhr0) het Missense NM_0010147.p.Nal25Mis GG7(At dhr0) het Missense NM_0010147.p.Hir35Arg (Gap (Aga dhr0) het Missense NM_001077.p.Hir921k aC(FA) dhr0) het Missense NM_001077.p.Hir921k aC(FA) dhr0) het Missense NM_001077.p.Hir921k aC(FA) dhr0) het Missense NM_001077.p.Gr921k aC(FA) dhr0 het Missense NM_0010577.p.Gly116Arg Gg7(Qg dhr1) het Missense NM_00008207.p.Gs93H1 Ta(Ac(dhr0) het Missense NM_00008207.p.Gs93H1 Ta(Ac(dhr0) het Missense NM_00008207.p.Sep3His C(Ac(dhr0) het Missense NM_00008207.p.Sep3His C(Ac(dhr0) het Missense NM_00008207.p.Sep3His C(Ac(dhr0) het Missense NM_00008207.p.Sep3H3 ta C(Ac(dhr1) het Missense NM_0000757.p.Gap1H3 ta C(Ac(dhr1) het Missense NM_0000757.p.Gap1H3$ | Patient_02 | NEB | Missense | NM_001164507:p.Asp5797Asn | Gat/Aat | chr02 | het |
| Patient_03 CTNNB1 Missense Missense NM_001098209p.C1394Arg, CG/A4 CG/A4 chr03 het Fallent_05 CG/A4 chr03 het het het het het Fallent_05 CG/A4 chr03 het het het het Fallent_05 CG/A4 chr03 het het het Fallent_04 Missense NM_001098209p.Ser37Pro Tcl/Cct chr03 het Fallent_05 Missense NM_00109747p.1hr912 cG/AG chr01 het Fallent_07 Missense NM_00109944p.Arg219Cys Cg/Tg chr05 het FH1011M Missense NM_0010757p.G311hArg Cg/CAG chr01 het FH101XM Missense NM_001077p.G311hArg Cg/CAG chr01 het FH101XM Missense NM_0010757p.G311hArg Cg/CAC chr01 het FH101XM Missense NM_00108209.p.5er37bc Cl/AC chr08 het FH101XM Misse | | SHPK | Missense | NM_013276:p.Glu477Asp | gaA/gaC | chr17 | het |
| Pathent_03 KCMF1 Missense NM_020122p-Ag257His' Cd/cAt chr02 het Patient_05 CTNNB1 Missense NM_001092429p-gc57Pro Tct/Cct chr03 het Patient_05 Missense NM_17834sp.Arg83His Cd/cAt chr01 het Patient_05 Missense NM_0101972p.Thr921le Cd/cAt chr01 het PAtient_10 Missense NM_010177511p.flic232ser Cd/cAt chr04 het PALU Missense NM_00105577.pc1011Arg Cgt/Cgt chr16 het PALU Missense NM_00105577.pc1011Arg Cgt/Cgt chr13 het Patient_07 Cforf2 Missense NM_010105577.pc1011Arg Cgt/Cgt chr13 het Patient_07 Missense NM_0010320_Arg84His CG/cAc chr08 het Patient_07 Missense NM_00108920_pc31420_Arg84His CG/cAc chr08 het Patient_08 MCX1 Missense NM_00108920_pc31420_Arg84His CG/cAc chr08 <td>D () ()</td> <td>CTNNB1</td> <td>Missense</td> <td>NM_001098209:p.Gly34Arg</td> <td>Gga/Aga</td> <td>chr03</td> <td>het</td> | D () () | CTNNB1 | Missense | NM_001098209:p.Gly34Arg | Gga/Aga | chr03 | het |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Patient_03 | KCMF1 | Missense | NM_020122:p.Arg257His | cGt/cAt | chr02 | het |
| $Patient_05 \begin{tabular}{l c c c c c c c c c c c c c c c c c c c$ | | CTNNB1 | Missense | NM 001098209:p.Ser37Pro | Tct/Cct | chr03 | het |
| $ \begin{array}{c ccccc} LCLF & Missense & NM_0178354_p.Arg83His & CGi/cAi & chr01 & het \\ IRCH & Missense & NM_00116211p.His545arg & cAi/CG & chr01 & het \\ NM_0110571p.D1hr92He & GC/AIC & chr01 & het \\ NM_01105571p.D1hr92He & GC/AIC & chr01 & het \\ PRD111 & Missense & NM_00109941p.Arg8494Cy & GC & GC & chr01 & het \\ PRD111 & Missense & NM_00105571p.G116Arg & Gg/Cgt & chr13 & het \\ NM_0025571p.G116Arg & Gg/Cgt & chr13 & het \\ NM_0003571p.G116Arg & Gg/Cgt & chr13 & het \\ NM_000312p.Arg8418His & CG/cAt & chr01 & het \\ ACTLS & Missense & NM_00312p.Arg8418His & CG/cAt & chr01 & het \\ ADCK5 & Missense & NM_00312p.Arg8418His & CG/cAt & chr03 & het \\ ADCK5 & Missense & NM_00312p.Arg8418His & CG/cAt & chr03 & het \\ ADCK5 & Missense & NM_00312p.Arg8418His & CG/cAt & chr03 & het \\ Missense & NM_0010850p.gsc33Cys & Cf/fd & chr14 & het \\ CTNNB1 & Missense & NM_001089091p.Sc33Cys & Cf/fd & chr15 & het \\ MDR62 & Missense & NM_001089091p.Sc33Cys & Cf/fd & chr12 & het \\ CTNNB1 & Missense & NM_001089091p.Sc33Cys & Cf/fd & chr12 & het \\ MAP2K1 & Missense & NM_001089091p.Sc33Cys & Cf/fd & chr12 & het \\ MAP2K1 & Missense & NM_001089091p.Sc33Cys & Cf/fd & chr12 & het \\ MAP2K1 & Missense & NM_00108209p.Sc37Phe & Cf/H1 & chr12 & het \\ NRC8 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ NAP2K1 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr14 & het \\ STG71 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ NRC1 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ STG71 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ STG71 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ CTNNB1 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ STG71 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ CTNNB1 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ CTNNB1 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ CTNNB1 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr14 & het \\ FGG7 & Missense & NM_001098209p.Sc37Phe & Cf/H1 & chr13 & het \\ CTNNB1 & Missense & NM_001098209p.Sc37Phe &$ | | DOCK8 | Missense | NM 203447:p.Val245Met | Gtg/Atg | chr09 | het |
| | | LCE1F | Missense | NM_178354;p.Arg83His | cGt/cAt | chr01 | het |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | | LRCH1 | Missense | NM_001164211:p.His745Arg | cAt/cGt | chr13 | het |
| Patient_05 PKD1 Missense NM_00100994tp_Argt249Cys Cgc/Tgc chr16 het PKHD1L Missense NM_002658p_His224Cin caC/caG chr08 het PLAU Missense NM_0010577p_Cp1His22352er aT/raGt chr01 het PRHOXIN Missense NM_01010577p_Cp1His224Cin caC/cAC chr17 het ACTL8 Missense NM_01010577p_Cp1His72 cGr/cAc chr01 het ADCK5 Missense NM_0106008p_Cy144Phis CG/cAc chr03 het Patient_07 Clorif2 Missense NM_00108200p_f5ca373Cu gG/gAs chr16 het CLIP1 Missense NM_00108200p_f5ca373Vp GL/r16 chr12 het WDR62 Missense NM_00108200p_f5ca37bh GL/r16 chr13 het MAP2X1 Missense NM_00108200p_f5ca37bh GL/r16 chr14 het Patient_08 RR466 Missense NM_0010927p_H1250Vaf530As Ga/cAa chr11 het | Detiont OF | N4BP2 | Missense | NM_018177:p.Thr92Ile | aCc/aTc | chr04 | het |
| $PkHD1L1 Missense NM_002685; His2232er a GT/aG chr08 het PLAU Missense NM_00105577; GY116Arg Ggt/Cgt chr13 het TUSC5 Missense NM_0123657; GY116Arg Ggt/Cgt chr13 het TUSC5 Missense NM_012367; Arg48His GG/AC chr08 het ARMCX1 Missense NM_0160695; Cy2r, Arg449His GG/AC chr08 het ARMCX1 Missense NM_0160695; Cy2r, Arg449His GG/AC chr08 het GC/AC chr08 het GC/CT2 Missense NM_0106095; Cy2r, Arg449His GG/AC chr08 het MR_00101571; Cloroff2 Missense NM_0102314; Arg48His GG/AC chr08 het MR_0016095; Cy2r, Arg449His GG/AC chr08 het MR_0016095; Cy2r, Arg449His GG/AC chr08 het MR_0016095; Cy2r, Arg449His GG/AC chr08 het MR_0010399; Ser33Cys GC/gAc chr12 het WDR62 Missense NM_0010399; Ser33Cys GC/gAc chr12 het WDR62 Missense NM_0010399; Ser37be Cl/Aft chr03 het MR_00124797; Diete30val Art/Gt chr03 het MR_00124797; Diete30val Art/Gt chr12 het GT/NB1 Missense NM_0010399; Ser37be Cl/Aft chr13 het MAP214 Missense NM_0010399; Ser37be Cl/Aft chr13 het SFTP1 Missense NM_0010375; Diete30val Art/Gt chr14 het SFTP1 Missense NM_0010375; Diete30val Art/Gt chr15 het SIC26A10 Missense NM_000372; Dia265Glu gCa/gAa chr11 het SIC7G1 Missense NM_000372; Dia265Glu GG/gAa chr11 het SIC7G1 Missense NM_001399; Ser37be Cl/Aft chr03 het SIC26A10 Missense NM_001399; Ser37be Cl/Aft chr18 het SIC26A10 Missense NM_001399; Ser37be Cl/Aft chr18 het SIC26A10 Missense NM_001399; Ser37be Cl/Aft chr18 het SIC26A10 Missense NM_000374; Dia265Glu gCa/gAa chr11 het SIC7G1 Missense NM_0013950; Arg4203Gh CGa/Aa chr11 het SIC26A10 Missense NM_0013950; Ser37be Cl/Aft chr18 het SIC26A10 Missense NM_0013950; Ser37be Cl/Aft chr14 het Cl/675 Missense NM_0013950; Ser37be Cl/Aft chr14 het Cl/676 Missense$ | ratient_05 | PKD1 | Missense | NM_001009944:p.Arg4249Cys | Cgc/Tgc | chr16 | het |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | | PKHD1L1 | Missense | NM_177531:p.Ile2532Ser | aTt/aGt | chr08 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | PLAU | Missense | NM_002658:p.His224Gln | caC/caG | chr10 | het |
| $ \begin{array}{c ccccc} TUSC5 & Missense & NM_102367p.5er937hr Tec/Acc & chr17 & het \\ \hline ACTL8 & Missense & NM_030612p.Arg48His & Cdr/Act & chr01 & het \\ ADCK5 & Missense & NM_01608p.Cys144Tyr & Cdr/Ac & chr08 & het \\ ADCK5 & Missense & NM_020314p.Ala33Gh & Cdr/Ac & chr08 & het \\ C160f62 & Missense & NM_00198209p.5er33Cys & Cdr/Ac & chr16 & het \\ CTTNB1 & Missense & NM_001098209p.5er33Cys & Cdr/Ac & chr12 & het \\ TTNB1 & Missense & NM_001098209p.5er37Che & Cdr/At & chr19 & het \\ MDR62 & Missense & NM_001098209p.5er37Che & Cdr/At & chr17 & het \\ MDR62 & Missense & NM_001098209p.5er37Che & Cdr/At & chr17 & het \\ MDR62 & Missense & NM_001098209p.5er37Che & Cdr/At & chr17 & het \\ MAP2K1 & Missense & NM_001098209p.5er37Che & Cdr/At & chr17 & het \\ MAP2K1 & Missense & NM_0000372tp.The420Fis & Cdr/Ac & chr17 & het \\ STRF1 & Missense & NM_000372tp.The426Giu & gCa/Aa & chr11 & het \\ STRF1 & Missense & NM_000372tp.Tha26Giu & gCa/Aa & chr11 & het \\ STRF1 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ STRF1 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ STRF1 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ DDX42 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ DX42 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ DX42 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ DX42 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ CTNNB1 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ DX42 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ CTNNB1 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ DX42 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ CTNNB1 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ C160r3 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ C160r3 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr03 & het \\ C160r3 & Missense & NM_001098209p.5er37Phe & tCt/tTt & chr04 & het \\ C160r3 & Missense & NM_0010198557p.C1939A1 & Att/Gt & chr14 & het \\ RXT16 & Missens$ | | PRHOXNB | Missense | NM_001105577:p.Gly116Arg | Ggt/Cgt | chr13 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | TUSC5 | Missense | NM_172367:p.Ser93Thr | Tcc/Acc | chr17 | het |
| $ \begin{array}{c} ADCK5 \\ ARMCX1 \\ Missense \\ NM_016068; Cys14H7y \\ CiGorf62 \\ CTZ \\ Missense \\ NM_001098209; Ser33Cys \\ CC1/GC \\ CTZ \\ Missense \\ NM_001098209; Ser33Cys \\ CC1/GC \\ cHr12 \\ Het \\ WDR2 \\ WIssense \\ NM_001098209; Ser37Phe \\ CT/CH \\ chr12 $ | | ACTL8 | Missense | NM_030812:p.Arg48His | cGt/cAt | chr01 | het |
| $Patient_07 C16orfd2 Missense NM_0106085p.Cys1414yr tCrVAc chrX het Patient_07 C16orfd2 Missense NM_006431p.Cly98Asp gCrVAc chr12 het CTNNB1 Missense NM_001083961p.Val4071le Gtt/Att chr12 het WDR62 Missense NM_001083961p.Val4071le Gtt/Att chr12 het CTNNB1 Missense NM_001247997.p.1le450Val Att/Ctt chr12 het MAP2K1 Missense NM_001247997.p.1le450Val Att/Ctt chr13 het MAP2K1 Missense NM_00109755p.Leu42His cTt/CAt chr15 het MAP2K1 Missense NM_00109755p.Leu42His cTt/CAt chr15 het MAP2K1 Missense NM_0010755p.Leu42His cTt/CAt chr17 het SETBP1 Missense NM_000275p.Jac42000 gCa/Aac chr11 het SETBP1 Missense NM_0010975p.Tr1327Cys tAt/Ctt chr12 het SETBP1 Missense NM_010559p.Tr1327Cys tAt/Ctt chr12 het SETBP1 Missense NM_010575p.Jac4800 gCa/Aac chr11 het SETBP1 Missense NM_010597p.Set37Phe Ct/Chr1 chr03 het SETBP1 Missense NM_010327p.Atg200Cln cCa/Caa chr08 het SNTG1 Missense NM_0103967p.Set37Phe Ct/Chr1 het Missense NM_0103967p.Set37Phe Ct/Chr1 het Missense NM_0103967p.Set37Phe Ct/Chr1 het Missense NM_0103967p.Set37Phe Ct/Chr1 het Missense NM_00103940p.Set37Phe Ct/Chr1 het BHMT Missense NM_001049800p.Set37Phe Ct/Chr1 het BHMT Missense NM_001049500p.Set37Phe Ct/Chr2 hhr0 het ELF7 Missense NM_00103940p.The100Al Att/Cdt chr12 het C150rf3 Missense NM_0011341p.Set73Cly Agc/Cgc chr16 het C150rf3 Missense NM_001141p.Set757Cly Agc/Cgc chr16 het C150rf3 Missense NM_00124189p.Lyse757Cly Agc/Cgc chr16 het C150rf3 Missense NM_00124189p.Lyse757Cly Agc/Cgc chr16 het C150rf3 Missense NM_0012418p.Spt37Jal ath16 het C150rf3 Missense NM_001244189p.Lyse757Lls Agc/Cgc chr17 het FCGP Missense NM_001257p.Cly905y Ggc/Tgc chr17 het FFINIMI-MFDC6 Missense NM_001575p.Cly95$ | | ADCK5 | Missense | NM_174922:p.Arg449His | cGc/cAc | chr08 | het |
| $Patient_0' Cloopfb2 Missense NM_0021314p-Ala3SGui gbcg/kp3g Chrlb het CCTNNB1 Missense NM_0001098204p-Ser33Cys Ct//Gt chrl3 het WDR62 Missense NM_001098204p-Ser33Cys Ct//Gt chrl3 het WDR62 Missense NM_001098204p-Ser37Phe Ct//Gt chrl3 het MAP2K1 Missense NM_001098204p-Ser37Phe Ct//Tt chrl3 het NM_021247997p-11e450Val Att//Ctt chrl5 het NEK8 Missense NM_001098204p-Ser37Phe Ct//Ct chrl1 het SETEP1 Missense NM_0010755p-Leu42His cTl/cct chrl1 het SETEP1 Missense NM_000275p-Ala25GU gCa/gAa chrl1 het SL26A10 Missense NM_000275p-Ala25GU gCa/gAa chrl1 het SL26A10 Missense NM_00027p-Ser37Phe Ct//Tt chrl3 het SL26A10 Missense NM_00027p-Ser37Phe Ct//Ct chrl1 het SL26A10 Missense NM_00027p-Ala25GU gCa/gAa chrl1 het SL26A10 Missense NM_00027p-1Ala25GU gCa/gAa chrl1 het SL26A10 Missense NM_0008209:p.Ser37Phe tCt//Tt chrl3 het SL26A10 Missense NM_00198209:p.Ser37Phe tCt/Tt chrl3 het SL26A10 Missense NM_00198209:p.Ser37Phe tCt/Tt chrl3 het MI_00198209:p.Ser37Phe tCt/Tt chrl3 het MI_00198209:p.Ser37Phe tCt/Tt chrl3 het MI_00198209:p.Ser37Phe tCt/Tt chrl3 het MI_00198209:p.Ser37Phe tCt/Tt chrl3 het MI_001998209:p.Ser37Phe tCt/Tt chrl3 het tLSP9X Missense NM_00119457:p.11e190Val Att/Gt chrX het tLSP9X Missense NM_001098209:p.Ser37Phe tCt/Tt chrl3 het tLSP9X Missense NM_00199320:p.Ser37Phe tCt/Tt chrl3 het tLSP9X Missense NM_00199320:p.Se$ | D /: / 07 | ARMCXI | Missense | NM_016608:p.Cys1441yr | tGc/tAc | chrX | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | Patient_07 | C160rf62 | Missense | NM_020314:p.Ala53Glu | gCg/gAg | chr16 | het |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | | CC12 | Missense | NM_006431:p.Gly98Asp | gGc/gAc | chr12 | het |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | | WDR62 | Missense | NM_001098209:p.Ser33Cys | tCt/tGt | chr03 | het |
| $ \begin{array}{c} CLP1 & Missense \\ CTNNB1 & Missense \\ CTNNB1 & Missense \\ MAP2K1 & Missense \\ NM_0010255:p.Leu42His \\ MAP2K1 & Missense \\ NM_010275:p.Leu42His \\ CTt/CAt \\ chr15 & het \\ MAP2K1 & Missense \\ NM_010174:p.Phe1048er \\ GTK/CAt \\ chr17 & het \\ ROM1 & Missense \\ NM_000327:p.Ala265Glu \\ gCa/gAa \\ chr11 & het \\ SETBP1 & Missense \\ NM_001555:p.Tyr1327Cys \\ dtyr \\ dtyr \\ SETBP1 & Missense \\ NM_010559:p.Tyr1327Cys \\ dtyr \\ dtyr \\ SETC1 & Missense \\ NM_0109820:p.Ser37Phe \\ dtyr \\$ | | WDR62 | Missense | NM_001083961:p. val40711e | Gtt/Att | chr19 | net |
| $ \begin{array}{c} C1NNB1 & Missense \\ MAP2K1 & Missense \\ NM_001098209:p.5er3/Phe \\ KC/LAt Ch15 het \\ NEK8 & Missense \\ NM_01004704:p.Phe104Ser \\ C1C \\ ROM1 & Missense \\ ROM1 & Missense \\ NM_001004704:p.Phe104Ser \\ FC \\ ROM1 & Missense \\ NM_010527:p.Alag25Ghu \\ gCa /gAa \\ chr11 het \\ het \\ SLC26A10 & Missense \\ NM_015559:p.Ty1327Cys \\ tAt/KGt \\ chr18 het \\ SLC26A10 & Missense \\ NM_01098209:p.Ser37Phe \\ CTNNB1 & Missense \\ NM_001098209:p.Ser37Phe \\ TBP \\ Missense \\ NM_001098209:p.Ser37Phe \\ TBP \\ Missense \\ NM_001098209:p.Ser37Phe \\ TBP \\ Missense \\ NM_001039590:p.Asn2098Ser \\ aAt/aGt \\ chr2 \\ chr06 het \\ CTNNB1 & Missense \\ NM_001098209:p.Ser37Phe \\ TBP \\ Missense \\ NM_001039590:p.Asn2098Ser \\ aAt/aGt \\ chrX \\ het \\ CTNNB1 & Missense \\ NM_001098209:p.Ser37Phe \\ TCC \\ CC \\ chr01 \\ het \\ CTNNB1 \\ Missense \\ NM_001039590:p.Ser37SPhe \\ TCC \\ chr01 \\ het \\ CTNNB1 \\ Missense \\ NM_0010139457:p.He190Val \\ Att/Cft \\ chrX \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C16orf3 \\ Missense \\ NM_001214:p.Ser75C1y \\ Agc/Cg \\ chr16 \\ het \\ C1NNB1 \\ Missense \\ NM_001224:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_001241:p.Phe873Val \\ Ga/Aa \\ chr19 \\ het \\ FCGBP \\ Missense \\ NM_0012957:p.Cly66Cys \\ Ggc/Agg \\ chr19 \\ het $ | | CLIP1 | Missense | NM_001247997:p.lle450Val | Att/Gtt | chr12 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | CTNNB1 | Missense | NM_001098209:p.Ser37Phe | tCt/fft | chr03 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | MAP2KI | Missense | NM_002755:p.Leu42His | cIt/cAt | chr15 | het |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$ | Deller 1 00 | NEK8 | Missense | NM_178170:p.Asp530Asn | Gac/Aac | chr17 | het |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | Patient_08 | DK4C6 | Missense | NM_000227m_Ala2(5Chr | t lc/tCc | cnr11 | net |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | SETRD1 | Missense | NM 015559:p Tur1227Cuc | gCa/gAa | chr18 | het |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$ | | SL C26A10 | Missense | NM 133489:p Val488Met | $C t \alpha / \Delta t \alpha$ | chr12 | het |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | | SNTG1 | Missense | NM 018967:p. Arg202Gln | cGa/cAa | chr08 | het |
| $ \begin{array}{c} \mbox{Cr1NvB1} & \mbox{Missense} & \mbox{NM_000320}; p.350711e & \mbox{Cr1} & \mbox{Cr1} & \mbox{Cr1} & \mbox{Lin} & \mbox{Lin}$ | | CTNNB1 | Missonso | NIM 001098209::::: Sor37Pho | +C+/+T+ | chr03 | hot |
| Patient_09DDATA TRPInscribe Missense $NM_003192P_1Thr106Ala$ $Mc03194P_TThr106Ala$ $Acg/GcgAcg/Gcgchr06hethetPatient_10CTNNB1FAM89AMissenseNM_001098209; p.Ser33PheMI_010198502; p.Ser175CystCt/tTttCt/tTtchr03hetPatient_10CTNNB1FAM89AMissenseNM_001198209; p.Ser33PheMI_001139457; p.Ile190ValAtt/Gttchr05hetBCAP31BHMTC160rf3C160rf3MissenseMissenseNM_001139457; p.Ile190ValMI_001214; p. Ser57ClyAgc/GgcAgc/GgcChr16hetC160rf3C160rf3MissenseNM_001214; p.Ser57ClyAgc/GgcCL60rf3AissenseNM_001214; p.Ser57ClyAgc/GgcChr16hetCRAMP1LCRAMP1LMissenseNM_001098209; p.Asp32TlyrGac/TacChr05hethetCRAMP1LE2F7FISSenseNM_001214; p.Ser57ClyAgc/GgcAgc/GgcChr16hetFCGBPFGGYMissenseNM_001098209; p.Asp32TlyrGac/TacChr03hetFCGBPFGGYMissenseNM_0012394; p.Phe873ValMisoenseNM_0012394; p.Phe873ValMisoensehetFGGYFGGYMissenseNM_00013980; p.Gly4778AspFGGYMissenseMM_0011341; p.Ser21AsnAa/aTaAa/aTaAhr14hetFGGYFGGYMissenseNM_00113412; p.Sys331leAa/aTaAa/aTaAhr14hetFGGYFGFYMissenseNM_00113412; p.Ser21AsnAa/aTaAa/aTaAhr14het$ | | | Missense | NM_007372:n Thr581Ala | Acc/Gcc | chr17 | het |
| $\begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Patient_09 | TRP | Missense | NM_003194:p Thr106Ala | Acg/Gcg | chr06 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | USP9X | Missense | NM_001039590:p.Asn2098Ser | aAt/aGt | chrX | het |
| Patient_10Driver FAM89AMissenseNM_198552:p.Ser175CysHCc/HGdriver HTdriver Het $BCAP31$ MissenseNM_001139457:p.Ile190ValAtt/Gttchr01het $BCAP31$ MissenseNM_0011713:p.Asp105AsnGac/Aacchr05het $C16orf3$ MissenseNM_001214:p.Val60IleGta/Atachr16het $C16orf3$ MissenseNM_001214:p.Ser57GlyAgc/Ggcchr16het $COL5A3$ MissenseNM_001214:p.Ser57GlyAgc/Ggcchr16het $CRAMP1L$ MissenseNM_00282p:p.ro818ThrCcc/Accchr16het $CRAMP1L$ MissenseNM_00109820:p:p.Asp32TyrGac/Tacchr03het $E2F7$ MissenseNM_00109820:p:DAsp32TyrGac/Tacchr03het $FCGBP$ MissenseNM_00113394:p.Phe873ValTtt/Gttchr11het $FGGY$ MissenseNM_00113411:p.Ser21AsnaGt/aAtchr01het $FGGY$ MissenseNM_001241:P.y.Ly953IleaAa/aTachr14het $FRT16$ MissenseNM_001241:P.y.Ly953IleaAa/aTachr14het $FT16$ MissenseNM_0012421:P.y.Ly953IleaAa/aTachr17het $Patient_11$ KELMissenseNM_017432:p.Ly973IleaAa/aTachr17het $FGP3$ MissenseNM_01122962:p.Gly94ArgGgg/Aggchr20het $FRP2$ MissenseNM_0112962:p.Gly11SerGgt/Agtchr02het $FGH4$ <td></td> <td>CTNNB1</td> <td>Missense</td> <td>NM_001098209:p.Ser33Phe</td> <td>tCt/tTt</td> <td>chr03</td> <td>het</td> | | CTNNB1 | Missense | NM_001098209:p.Ser33Phe | tCt/tTt | chr03 | het |
| $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | Patient_10 | FAM89A | Missense | NM_198552:p.Ser175Cys | tCc/tGc | chr01 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | BCAP31 | Missense | NM_001139457:p.Ile190Val | Att/Gtt | chrX | het |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | | BHMT | Missense | NM_001713:p.Asp105Asn | Gac/Aac | chr05 | het |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | | C16orf3 | Missense | NM_001214:p.Val60Ile | Gta/Ata | chr16 | het |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | | C16orf3 | Missense | NM_001214:p.Ser57Gly | Agc/Ggc | chr16 | het |
| $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | | COL5A3 | Missense | NM_015719:p.Gly533Val | gGa/gTa | chr19 | het |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | | CRAMP1L | Missense | NM_020825:p.Pro818Thr | Ccc/Acc | chr16 | het |
| $E2F7$ MissenseNM_203394:p.Phe873ValTtt/Gttchr12het $FCGBP$ MissenseNM_003890:p.Gly4778AspgGc/gAcchr19het $FGGY$ MissenseNM_001113411:p.Ser21AsnaGt/aAtchr01hetPatient_11KELMissenseNM_000420:p.Arg393GlncGg/cAgchr07het $KIAA0586$ MissenseNM_001244189:p.Lys953IleaAa/aTachr14het $KRT16$ MissenseNM_005557:p.Gly69CysGgc/Tgcchr17het $PEX10$ MissenseNM_0153818:p.Leu221HiscTc/cAcchr01het $PTOV1$ MissenseNM_001122962:p.Gly94ArgGgg/Aggchr20het $SPINLW1-WFDC6$ MissenseNM_00119896:p.Glu141LysGaa/Aaachr02het $THAP4$ MissenseNM_0019533:p.Gly111SerGgt/Agtchr02het $TMEM99$ MissenseNM_001195386:p.Asp195AsnGac/Aacchr17het TYR MissenseNM_000372:p.Thr292MetaCg/aTgchr11het $UMODL1$ MissenseNM_001372:p.Thr292MetaCg/aTgchr11het | | CTNNB1 | Missense | NM_001098209:p.Asp32Tyr | Gac/Tac | chr03 | het |
| FCGBPMissenseNM_003890:p.Gly4778AspgGc/gAcchr19hetFGGYMissenseNM_001113411:p.Ser21AsnaGt/aAtchr01hetPatient_11KELMissenseNM_000420:p.Arg393GlncGg/cAgchr07hetKIAA0586MissenseNM_001244189:p.Lys953lleaAa/aTachr14hetKRT16MissenseNM_0015557:p.Gly69CysGgc/Tgcchr17hetPEX10MissenseNM_0153818:p.Leu221HiscTc/cAcchr01hetPTOV1MissenseNM_017432:p.Lys912MetaAg/aTgchr19hetSIRPB2MissenseNM_00112962:p.Gly94ArgGgg/Aggchr20hetTHAP4MissenseNM_00119896:p.Glu141LysGaa/Aaachr02hetTMEM99MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_000372:p.Thr292MetaCg/aGGchr21het | | E2F7 | Missense | NM_203394:p.Phe873Val | Ttt/Gtt | chr12 | het |
| FGGYMissenseNM_001113411:p.Ser21AsnaGf/aAtchr01hetPatient_11KELMissenseNM_000420:p.Arg393GlncGg/cAgchr07hetKIAA0586MissenseNM_001244189:p.Lys953lleaAa/aTachr14hetKRT16MissenseNM_005557:p.Gly69CysGgc/Tgcchr17hetPEX10MissenseNM_005557:p.Gly69CysGgc/Tgcchr19hetPTOV1MissenseNM_017432:p.Lys212MetaAg/aTgchr19hetSIRPB2MissenseNM_00112962:p.Gly94ArgGgg/Aggchr20hetSPINLW1-WFDC6MissenseNM_00119896:p.Glu141LysGaa/Aaachr20hetTHAP4MissenseNM_00119538:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | FCGBP | Missense | NM_003890:p.Gly4778Asp | gGc/gAc | chr19 | het |
| Patient_11KELMissenseNM_000420:p.Arg393Gin cGg/cAg $chr07$ het $KIAA0586$ MissenseNM_001244189:p.Lys953lle aAa/aTa $chr14$ het $KRT16$ MissenseNM_0015557:p.Gly69CysGgc/Tgc $chr17$ het $PEX10$ MissenseNM_153818:p.Leu221His cTc/cAc $chr19$ het $PTOV1$ MissenseNM_0017432:p.Lys912Met aAg/aTg $chr19$ het $SIRPB2$ MissenseNM_00112962:p.Gly94ArgGgg/Agg $chr20$ het $SPINLW1-WFDC6$ MissenseNM_001198986:p.Glu141LysGaa/Aaa $chr20$ het $THAP4$ MissenseNM_001195363:p.Gly111SerGgf/Agtchr02het $TMEM99$ MissenseNM_001195386:p.Asp195AsnGac/Aacchr17het TYR MissenseNM_000372:p.Thr292Met aCg/aTg chr11het $UMODL1$ MissenseNM_173568:p.Asp814Glu gaC/gaG chr21het | D (1 / 11 | FGGY | Missense | NM_001113411:p.Ser21Asn | aGt/aAt | chr01 | het |
| KIAA0350Missense $NM_001244189:p.Lys955ileaAa/a1achr14hetKRT16MissenseNM_005557:p.Gly69CysGgc/Tgcchr17hetPEX10MissenseNM_005557:p.Gly69CysGgc/Tgcchr17hetPTOV1MissenseNM_017332:p.Lys212MetaAg/aTgchr19hetSIRPB2MissenseNM_001122962:p.Gly94ArgGgg/Aggchr20hetSPINLW1-WFDC6MissenseNM_001198986:p.Glu141LysGaa/Aaachr20hetTHAP4MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het$ | Patient_11 | KEL | Missense | NM_001244190 | cGg/cAg | chrU/ | net |
| NKI 10MissenseNMI_00337:p.Gly09CysGgC/1gcChr17hetPEX10MissenseNM_00337:p.Gly09CysCfc/cAcchr19hetPTOV1MissenseNM_017432:p.Lys212MetaAg/aTgchr19hetSIRPB2MissenseNM_001122962:p.Gly04ArgGgg/Aggchr20hetSPINLW1-WFDC6MissenseNM_001198986:p.Glu141LysGaa/Aaachr20hetTHAP4MissenseNM_015963:p.Gly111SerGgt/Agtchr02hetTMEM99MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | KIAAU586 | Missense | NIM 005557 | aAa/ala | cnr14 | net |
| PENDMissenseNM_00372:p.Lys212MetaAg/aTgchr01hetPTOV1MissenseNM_001122962:p.Gly94ArgGgg/Aggchr02hetSIRPB2MissenseNM_00112986:p.Glu141LysGaa/Aaachr20hetSPINLW1-WFDC6MissenseNM_015963:p.Gly111SerGgt/Agtchr02hetTHAP4MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | NKI 10 DEV10 | Missense | NIVI_000007:p.GIY69Cys | GgC/1gC | chr01 | het |
| SIRPB2MissenseNM_001122962:p.Gly94ArgGgg/Aggchr19InterSPINLW1-WFDC6MissenseNM_001198986:p.Glu141LysGaa/Aaachr20hetTHAP4MissenseNM_015963:p.Gly111SerGgt/Agtchr02hetTMEM99MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | FEAIU PTOV1 | Missense | NM 017432 D I wo212Mot | $\alpha \alpha / \alpha T \alpha$ | chr10 | het |
| SPINLW1-WFDC6MissenseNM_00112292.p.Gly34AigGgg/AggCli 20InterSPINLW1-WFDC6MissenseNM_001198986:p.Glu141LysGaa/Aaachr20hetTHAP4MissenseNM_015963:p.Gly111SerGgt/Agtchr02hetTMEM99MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | SIRDRO | Missense | NM 001122962 m Cly04 Are | ang/aig | chr20 | het |
| THAP4MissenseNM_01190305.p.Gly111SerGgt/Agtchr02hetTMEM99MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | SPINIW1_WFDC6 | Missense | NM 001198986 n Clu1411 ve | Gag/Agg | chr20 | het |
| TMEM99MissenseNM_001195386:p.Asp195AsnGac/Aacchr17hetTYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | THAP4 | Missense | NM 015963 n Glv111Ser | Got/Aot | chr02 | het |
| TYRMissenseNM_000372:p.Thr292MetaCg/aTgchr11hetUMODL1MissenseNM_173568:p.Asp814GlugaC/gaGchr21het | | TMEM99 | Missense | NM 001195386 n Asp195Asp | Gac/Aac | chr17 | het |
| UMODL1 Missense NM_173568:p.Asp814Glu gaC/gaG chr21 het | | TYR | Missense | NM 000372:p.Thr292Met | aCg/aTg | chr11 | het |
| | | UMODL1 | Missense | NM_173568:p.Asp814Glu | gaC/gaG | chr21 | het |

Table 2. Cont.

Codon: Capital letter represents the variational base and lowercase represents the uniformity.



Figure 1. Single nucleotide polymorphism (SNP) distributions in solid pseudopapillary tumor of the pancreas. (**A**) The overview of non-synonymous mononucleotide variation corresponding to each samples. White and light yellow indicate the low and moderate variations count, respectively; Dark and brownish yellow indicate the multitude variations count, respectively; (**B**) SNP events distributed in each patient; (**C**) SNPs events distributed in each chromosome.

2.2. Insertions and Deletions in SPT

In total, 56 significant insertions and deletions (indels) in the DNA were detected in the nine subjects, and 41 known genes were associated with those indels (Table 3). Functional annotation showed that a number of (25 of 56) indels would introduce a frame shift, and two indels would generate a splicing alteration (Figure 1A). We predicted that the impact of these sequence changes, 27 indels showing frame shift and/or splicing site changes, might be important in the biological activity of the cell, and that the other 29 events might play secondary roles (Figure 2B). Chromosome distribution showed that the regions of high impact were mostly located in chromosomes 19 and 20 (Figure 2C). We also compared the genes involved in indels and SNPs, and only one common gene, TBP (TATA-box binding protein), was detected.

| Table 3. Impact and functional annota | ations of detected Indel variations. |
|---------------------------------------|--------------------------------------|
|---------------------------------------|--------------------------------------|

| Impact | Function | Chr | Gene | Reference | Observation | Alleles |
|--------|----------|-------|--------|-----------|-------------|---------|
| High | FS | chr01 | AHDC1 | Т | TG | het |
| High | FS | chr01 | LRRIQ3 | G | GT | het |
| High | FS | chr10 | NOC3L | AT | А | het |
| High | FS | chr10 | TFAM | CA | С | het |
| High | FS | chr12 | TDG | G | GA | het |
| High | FS | chr14 | CCNK | G | GC | het |
| High | FS | chr14 | PAPOLA | TG | Т | het |
| High | FS | chr16 | IRX5 | AGG | А | het |
| High | FS | chr17 | ACSF2 | Т | TAA | het |

| Impact | Function | Chr | Gene | Reference | Observation | Alleles |
|-----------|----------|--------|-----------|----------------------|------------------|---------|
| High | FS | chr17 | KRT10 | CCGCCG | С | het |
| High | FS | chr17 | KRT10 | TG | Т | het |
| High | FS | chr19 | CAPN12 | G | GC | het |
| High | FS | chr19 | KCNC3 | С | CG | het |
| High | FS | chr02 | SNED1 | А | AC | het |
| High | FS | chr20 | C20orf132 | GACCT | G | het |
| High | FS | chr20 | C20orf132 | GC | G | het |
| High | FS | chr20 | C20orf132 | GAGGAGTT | G | het |
| High | FS | chr20 | C20orf132 | CG | С | het |
| High | FS | chr20 | C20orf132 | TGG | Т | het |
| High | FS | chr06 | TBP | AGC | А | het |
| High | FS | chr06 | TBP | AG | А | het |
| High | FS | chr06 | TFB1M | CAA | С | het |
| High | FS | chr09 | PHF2 | А | AG | het |
| High | FS | chrX | PLXNA3 | Т | TG | het |
| High | FS | chrX | RBM10 | CA | С | het |
| High | SSD | chr19 | KRI1 | CCATCA | С | het |
| High | SSD | chr19 | KRI1 | CCATCA | С | het |
| Moderate | C & D | chr11 | SCUBE2 | GGCA | G | het |
| Moderate | C & D | chr12 | ATXN2 | GGCT | G | het |
| Moderate | C & D | chr14 | MAP3K9 | GCCT | G | het |
| Moderate | C & D | chr19 | SAFB2 | GTAC | G | het |
| Moderate | C & D | chr02 | GIGYF2 | CACA | С | het |
| Moderate | C & D | chr09 | TPRN | TTCC | Т | het |
| Moderate | C & D | chrX | AR | AAGAGACT AGCCCCAG | А | het |
| Moderate | C & I | chr11 | KRTAP5-8 | Т | TCCG | het |
| Moderate | C & I | chr14 | ATXN3 | С | CCTG | het |
| Moderate | C & I | chr14 | IRF2BPL | С | CTGCTGT | het |
| Moderate | C & I | chr04 | HTT | А | AACAGCC | het |
| Moderate | C & I | chr08 | ATAD2 | A TGGGACAGC | ATCG | het |
| Moderate | CD | chr16 | APOBR | CTCAGGAGGG | Т | het |
| Moderate | CD | chr17 | VDM6P | TCAC | т | hot |
| Moderate | CD | chi 17 | MPD2 | CCCA | | het |
| Moderate | CD | chr10 | | CGCA | C | het |
| Madarata | CD | chr04 | | GTGACACCC | G | hat |
| Wioderate | CD | cnr04 | ADAM29 | CAACCTCAGT | G | net |
| Moderate | CD | chr06 | KCNQ5 | AGCG | A | het |
| Moderate | CD | chr06 | TBP | GCAA | G | het |
| Moderate | CD | chr09 | RNF20 | GAAGACTCA | Т | het |
| Moderate | CI | chr10 | C10orf140 | С | CCCTCCT | het |
| Moderate | CI | chr12 | EP400 | А | ACAG | het |
| Moderate | CI | chr12 | EP400 | А | ACAG | het |
| Moderate | CI | chr12 | EP400 | G | GCAA | het |
| Moderate | CI | chr17 | KRTAP4-5 | Т | TGGCAGCAGCTGGGGC | het |
| Moderate | CI | chr19 | ZNF814 | С | CATA | het |
| Moderate | CI | chr04 | HTT | А | ACCGCCGCCG | het |
| Moderate | CI | chr06 | TBP | А | ACAG | het |
| Moderate | CI | chr06 | TBP | А | ACAG | het |

Table 3. Cont.

FS: frame shift, SSD: splice site donor, CD: codon deletion, CI: codon insertion, G & I: codon change plus codon insertion, G & D: codon change plus codon deletion, Chr: chromosome.





2.3. The Network of Indels and Single Nucleotide Polymorphisms (SNPs) Related Genes

In neoplasm progression, indels and SNPs cause gene functional variation [10], and gene expression also regulates important cellular activities. We compared the combined set of gene variations with previously reported abnormally expressed genes [5] in SPT, and the results showed an overlap of two

genes, *CTNNB1* and *AR* (Figure 3A, bottom Venny schedule). Additionally, *CTNNB1* had the highest rate of variation events in the combined set. (Figure 3B). Phosphoproteins was shown as the biggest cluster based on the functions and pathway correlations (Figure 3C). Details of each cluster are listed in Table 4.



Figure 3. Combined set of variated genes: (**A**) Comparison of indels with SNPs involved genes (**top**) and present combined set with previously reported abnormally expressed genes (**bottom**) in SPN; (**B**) the variation events count of each homologous gene; (**C**) functions and pathways enrichment of combined variation events; and (**D**) network analysis according to String database.

| Category | Term | Count | Genes | Beniamin | FDR |
|---------------------|---|-------|--|----------------------|----------------------|
| Up keywords | Phosphoprotein | 56 | PLXNA3, KCNC3, TUSC5, E2F7, CCT2, CTNNB1, KCNQ5, MAP3K9, H2AFX, RBM10, AR, CCNK, C16ORF62, MED12, KRT10, KIAA0586, MBD2, LRCH1, KRT16, BHMT, NEK8, CLIP1, UNC13C, RNF20, GIGYF2, EP400, KDM6B, PTOV1, IRX5, THAP4, KEL, USP9X, NOC3L, N4BP2, TFAM, AP0BR, PKD1, KRI1, DDX42, MAP2K1, HTT, MY01E, ARID3A, ATAD2, DOCK8, SAFB2, ATXN2, ATXN3, PAP0LA, PHF2, KCMF1, WDR62, IRF2BPL, PLAU, TPRN, AHDC1 | 0.004376 | 0.086018 |
| Up keywords | Coiled coil | 29 | LRRIQ3, THAP4, NOC3L, TBP, N4BP2, MAP3K9, PKD1, KRI1, DDX42, AR, MAP2K1, HTT, ATAD2, KRT10, KIAA0586, BCAP31, ATXN2, ATXN3, PHF2, KCMF1, LRCH1, IRF2BPL, KRT16, CLIP1, UNC13C, RNF20, GIGYF2, EP400, TPRN | 0.003907 | 0.102373 |
| Up seqfeature | Compositionally biased region: Poly-Gln | 10 | ATXN2, CCNK, AR, ATXN3, KCNC3, IRF2BPL, HTT, KIAA0586, TBP, EP400 | $1.95 	imes 10^{-5}$ | $4.58 	imes 10^{-5}$ |
| Up keywords | Mental retardation | 10 | IRX5, MAP2K1, WDR62, USP9X, SETBP1, MED12, DOCK8, CTNNB1, AHDC1, BCAP31 | $6.05 	imes 10^{-4}$ | 0.007916 |
| Goterm mf direct | GO:0003682 ~chromatin binding | 10 | TFAM, AR, NOC3L, ARID3A, MED12, ATAD2, MBD2, RNF20, EP400, KDM6B | 0.022881 | 0.147425 |
| Up keywords | Triplet repeat expansion | 6 | ATXN2, AR, ATXN3, IRF2BPL, HTT, TBP | $3.76 	imes 10^{-5}$ | $2.46 	imes 10^{-4}$ |

| 1 |
|----------|
|----------|

FDR: false discover rate.

According to annotating protein–protein interaction using String database, *CTNNB1* was shown as a hub and directly connected with another six genes with a high confidence (score > 0.90) (Figure 3D). PKD1 (Protein Kinase D1), a serine-threonine kinase, has been reported to modulate the β -catenin functions in colon cancer [11]. The deubiquitination protein USP9X was shown to be required for lymphocyte activation [12]. EP400 is an E1A binding protein and deposits the histone variant H3.3 into chromatin alongside histone H2AZ and contributes to gene regulation [13]. The *HTT* gene coding for Huntington protein, is mutated in Huntington's disease but is ubiquitously expressed, and mutant *HTT* also influences cancer progression [14]. Additionally, other protein–protein connections such as KCNC3 vs. KCNQ5, ATXN3 vs. ATXN2, TFAM vs. TFB1M, and FGGY vs. SHPK also showed stronger paired connections.

3. Discussion

The low incidence of solid pseudopapillary tumor of the pancreas determined that large-scale susceptibility gene screening was unachievable. To explore the potential pathogenic gene, we describe here the first paired whole genome sequencing of SPT in the Chinese population with a limited sample size (nine neoplasm tissues vs. nine adjunct tissues). Our data revealed that multiple protein-coding related variations participated in SPT disease progression. However, gene variation distributions in each case are widely divergent. Even though *CTNNB1* mutations were detected throughout all patients, the mutated nucleic acid sites were different (Table 2). Those diversified variations suggested that SPT is a multi-heterogeneity disease, which might be caused by the dysregulation in the development of pancreas.

The function network suggests that *CTNNB1* may work as a hub and be closely connected with other gene variations, such as *USP9X*, *EP400*, *PDK1*, *MED12*, *HTT* and *AR*. Some of those genes have been reported to play an important role in other cancers [15–17]. Both indels and SNP sets showed TBP (TATA-box binding) dysfunction (Figure 1A), and this might cause the hallmarks of oncogene-induced replication stress, including replication fork slowing, DNA damage, and senescence [18]. Comparing

similarities of gene abnormality with former expression data [5], we noticed that CTNNB1 and AR (androgen receptor) were in the intersection, suggesting that AR signaling was also closely related [19]. Most colon cancer development and progression is involved in dysregulation of the β -catenin signaling pathway, and PKD1 was previously reported to directly interact with β -catenin, and to attenuate β -catenin transcriptional activity by decreasing nuclear β -catenin levels, which eventually suppressed colon cancer growth [11].

As the core portion in the co-connected network and the focus of multiple studies, the Wnt/ β -catenin (*CTNNB1* coding) pathway played an important role to facilitate carcinogenesis through regulated or unregulated changes in gene transcription [4,7,20,21]. Although considerable detail had revealed that the upstream factors induced activation of β -catenin in the cytoplasm, the mechanism by which β -catenin is involved with connected gene variations in different neoplasms is much less known [22]. In this study, we detected that β -catenin was mutated in all neoplasms studied (100%), and that this frequency was higher than previously reported (approximate 90%), suggesting that *CTNNB1* mutation is ubiquitous in SPT patients. The detection level may depend on detection methods. Moreover, functionally associating or physically binding with other candidates indicated that the effect of β -catenin might require assistant factors [15,23].

Among the studied patients, only patients 5 and 11 showed a distant metastasis phenotype. We detected many more SNPs were distributed in the metastatic disease compared to non-metastatic cases. Although this interesting phenomenon requires extended study, it suggests that enriched mutation might accelerate metastatic disease. Analogously, enriched mutation was also potentially related to larger tumor size. These discoveries have not been reported previously.

Based on functional annotations of indels adding SNPs genes, phosphoproteins were shown as the biggest cluster, revealing that most protein variations participated in signaling transduction. For instance, USP9x, a deubiquitinase, and connected with CTNNB1 as shown in the network, has been reported to be required for PKC β kinase activity and induced the cell survival and tumor-promoting activities of Notch signaling in cancer [12,24]. Additionally, significant enrichment of candidates also indicates an involvement in coiled-coil protein and mental retardation, suggesting the variation might cause structural abnormality and nervous system metastasis. Distinguishing with most previously study, we investigated the broad spectrum genes variations in SPT. All detected genetic variations need to be further verified.

4. Materials and Methods

4.1. Patients and Tissues

Eleven patients diagnosed with SPT and who underwent radical surgery in Shanghai Cancer Center, Fudan University (Shanghai, China), between 2010 and 2014 were selected for this study. SPT is circumscribed, solid, cystic masses and the pathology microscopic characteristic is typical pseudopapillae composed of central fibrovascular stalks embosomed by discohesive tumor cells with monotonous nuclei, absent nuclear pleomorphism, and low mitotic activity (Figure S1) [25,26]. The diagnoses of the resected tissues were confirmed by the Department of Pathology and 2 specimens (patient number 4 and patient number 6) were excluded because of the limited content of tumor cells (<30%). Clinical information regarding patient age, gender, TNM stage, tumor size, tumor location, and metastatic of non-metastatic disease, were collected from medical record files. TNM staging of each patient was based on AJCC (American Joint Committee on Cancer) classification criterion. Paired carcinoma and adjacent tissue specimens from the patients were frozen in liquid nitrogen and then store at -80 °C. The study was approved by the ethics committee of Fudan University Shanghai Cancer Center (ethical approval number: 050432-4-1212B, ethical approval date: 24 December 2012). Before the project began, written informed consent from all 9 patients was obtained, and the clinical events were evaluated based on the original histopathology reports and clinical records.

4.2. DNA Extraction and Exome Sequencing

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Before DNA Extraction, frozen sections of each tissue were stained with H&E to ensure the tumor cell number was more than 25% in the tissue. Genomic DNA from 9 tissues from each patient was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The exome of the genomic DNA was captured and sequenced using Agilent SureSelect system (BGI Co., Shenzhen, China) according to the manual. The DNA sample of genomic was fragmented randomly. The 150- to 200-bp fragments was utilized for the library and the adaptors were subsequently ligated to the fragments at both ends. The adapter-ligated templates were purified according to Agencourt AMPure SPRI beads (Beckmancoulter, Brea, CA, USA). For enrichment, the extracted DNA was amplified by LM-PCR (ligation-mediated PCR), purified, and hybridized to the SureSelect Biotinylated RNA Library (BAITS) (ABI, Waltham, MA, USA). After 24 h incubation, hybridized fragments were bound to the streptavidin beads whereas non-hybridized fragments were washed out. To estimate the magnitude of enrichment, captured LM-PCR products were analyzed by Agilent 2100 Bioanalyzer (Agilent Technologies, San Jose, CA, USA). Subsequently, the captured library was loaded on a Hiseq2000 platform (Illumina, San Diego, CA, USA) and sequenced in high-throughput with depth of more than $100 \times$ to ensure that each sample met the desired average sequencing depth. Raw image files were processed by Illumina basecalling Software 1.7 (Illumina) and the sequences information were generated as 90/100 bp pair-end reads. Representative variations of SNPs and indels were subsequently validated by Sanger sequencing (Figure S2).

4.3. Read Mapping and Standard Bioinformatics Analysis

The sequencing data (raw data) generated from the Illumina software (Illumina basecalling Software 1.7) was needed to conduct cleaning and mapping. The adapter sequence in the raw data and low quality sequences which had too many unknown bases or low base quality were excluded. Clean data was produced and aligned by BWA (http://biobwa.sourceforge.net/) and formatted the sequence into binary BAM files. The BAM format files were established mate information of the alignment, added read group information and removed duplicate reads caused by PCR. Clean reads were processed by mapped to the reference human genome (GRCh37/hg19) from UCSC database (http://genome.ucsc.edu/) using SOAPalinger (http://soap.genomics.org.cn/index.html). Single Nucleotide Polymorphisms (SNPs) were detected according to SOAPsnp (http://soap.genomics. org.cn/soapsnp.html). Indels were aligned to the reference human genome from UCSC using BWA and further conduct with the Genome Analysis Toolkit (GATK v1.6) for recalling. Variants in the non-coding region and synonymous mutations were removed. SNPs and indels with higher frequency (>0.5%) noted in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes (ftp://www.1000genome.org), HapMap were also filtered out. Quality Control (QC) was processed in the steps of the clean data, the alignment, and the identified variant.

4.4. Exome Homozygosity Mapping

Large stretches of the homozygous region were detected using the whole genome sequencing data. As markers to create a genetic map, all the autosomal dbSNP sites and novel SNPs that had \geq 20-fold coverage of the exome target regions were examined. For the homozygous markers selection, variants with \geq 95% of all reads displaying an identical SNP allele and covering at least 5-fold of the region were taken into consideration; for heterozygous markers selection, SNPs with 30% to 70% of all variation reads and which covered at least 10-fold were taken into consideration. The other SNPs with <30% or with 70%–95% variation reads were considered ambiguous. Perl script was utilized for statistical analysis of the distribution of map markers along the genome. A window of 500 markers, containing a maximum of 2 heterozygous markers and allowing a maximum gap between 2 adjacent markers of 500 KB, was adopted. A homozygous stretch by coalescence of all qualified windows with a minimum of 1 MB in length identified as a genomic region.

4.5. Cluster and Network

Each of the genes detected in the neoplasms with prominent SNPs and indels was functionally annotated and clustered by David database (https://david.ncifcrf.gov). After importing the genes list into String database (http://www.string-db.org), the high confidence (>0.7) connection between genes was presented, which might be co-mentioned or mutually bound.

4.6. Statistical Analysis

Statistical analysis was performed using SPSS software (v13.0, Chicago, IL, USA). Data were analyzed and statistically assessed by Fisher's exact tests. p < 0.01 was considered to be significant for all statistical analyses.

5. Conclusions

In the current study, we conducted whole exome sequencing in 9 SPT patients, which detected 54 SNPs and 41 indels of prominent variations in total. Multiple SNPs with a higher count was found to correlate with adverse clinical manifestations. In addition to be detected throughout all cases, *CTNNB1* mutation was presented to potentially collaborate with other gene variations. The aberration events involved in other cancers also showed the potential to stimulate the progression of SPT. This work revealed an insight into the variation of the gene encoding regions might partly reflect the potential molecular mechanism of SPT.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/1422-0067/18/1/81/s1.

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Author Contributions: Xianjun Yu and Guopei Luo conceived and designed the project; Jiang Long collected and processed the samples; Chen Liu analyzed the data; Kaizhou Jin, He Cheng and Yu Lu contributed analysis and diagram; Zhengshi Wang and Chao Yang provided technical support; Jin Xu and Quanxing Ni provided clinical counseling; and Meng Guo wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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