Chapter 4 Characterisation of *las1l*^{sa674} mutants

4.1 Introduction

In the previous chapter, Nol9 was identified as playing a role in pancreas development in zebrafish. In this chapter, I aimed to investigate whether any Nol9-interacting proteins had a similar function to Nol9. A literature search revealed that the nucleolar protein LAS1-like (LAS1L) forms a complex with proteins Nucleolar Protein 9 (NOL9), SUMO1/sentrin/SMT3 specific peptidase 3 (SENP3), Proline, glutamic acid and leucine rich protein 1 (PELP1), Testis expressed 10 (TEX10) and WD repeat domain 18 (WDR18) (Castle et al., 2012). LAS1L and its associated proteins are involved in processing of the pre-rRNA internal transcribed spacer 2 region to produce 28S rRNA (Castle et al., 2012; Castle et al., 2010). Las1, the yeast orthologue of LAS1L, was initially found to be involved in cell morphogenesis and also to play a conserved role in processing of rRNAs of the large ribosomal subunit (Castle et al., 2013; Doseff and Arndt, 1995). Recently, Butterfield *et al.* described a patient with a spinal muscular atrophy with respiratory distress (SMARD) phenotype and identified a de novo mutation in *LAS1L* (Butterfield et al., 2014). The authors knocked down *las1l* in zebrafish by morpholino resulting in disruption of peripheral nerve and muscle architecture and early lethality of the embryos (Butterfield et al., 2014). There are currently no reports of studies of LAS1L in mice. The Zebrafish Mutation Project was searched for disruptive mutations in *las1l*, *senp3a*, *senp3b*, *PELP1*, *tex10* and *wdr18*, zebrafish orthologues of *LAS1L*, *SENP3*, *PELP1*, *TEX10* and *WDR18*. At the time of the search, a nonsense allele was identified for *las1l* (*las1l*^{sa674}), whereas alleles of *senp3a*, *senp3b*, *PELP1*, *tex10* and *wdr18* had not yet been found.

The *las1l* gene has three Zv9 Ensembl transcripts: las1l-001, las1l-201 and las1l-202 (Figure 4-1 A). The longest transcript las1l-001 is comprised of 2642 bases and has 13 exons encoding a protein of 580 amino acids. The las1l-201 and las1l-202 are two shorter transcripts comprising of 942 and 567 bases encoding a protein of 314 and 189 amino acids, respectively. The Las1l protein of human (ENSP00000363944), mouse (ENSMUSP00000078901) and zebrafish (ENSDARP00000121984) are conserved especially

in the *Las1* domain (Figure 4-1 B). The zebrafish Las1l has 37% and 38% amino acid residues that are identical to the human and mouse Las1l respectively. The *las1l*^{sa674} has a G to T base change in exon 11 of the transcript las1l-001 converting the amino acid 475 from a glutamic acid (GAG) into a stop codon (TAG) (Figure 4-1 A, B).

In order to determine whether Las1l has a similar function to Nol9 in zebrafish development, I aimed to characterise the *las1l*$^{sa674/sa674}$ mutant (abbreviated as *las1l*^{sa674}) by first identifying any gross morphological defects that may be present in *las1l*^{sa674} mutant, focusing on the digestive organs since they are hypoplastic in *nol9*^{sa1022} mutant. Secondly, I aimed to determine whether the pancreatic defects of *las1l*^{sa674} mutant were comparable to those of the *nol9*^{sa1022} mutant by identifying the stage at which pancreas development is impaired. Thirdly, I analysed cell death in *las1l*^{sa674} mutant in order to investigate whether increased cell death could contribute to the phenotype. Lastly, I examined the jaw morphology and erythrocyte development of *las1l*^{sa674} mutant to determine whether they were normal and similar to the *nol9*^{sa1022} mutant.

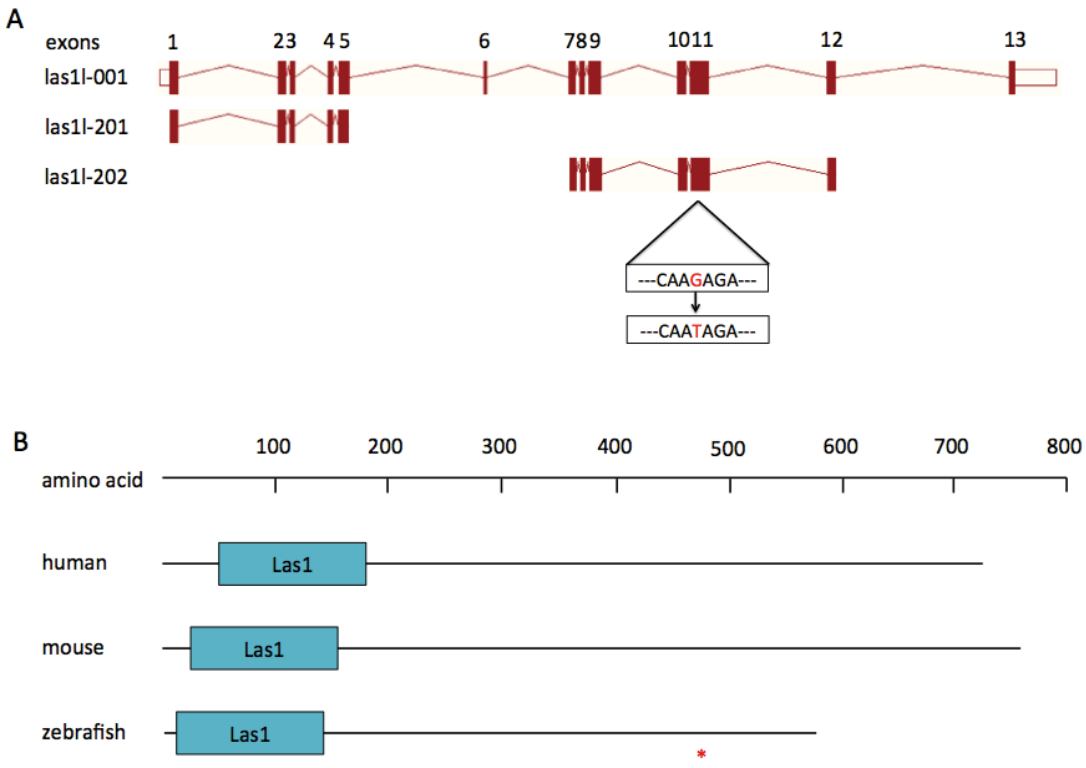

Figure 4-1 Diagram of *las1l* gene and Las1l protein. (A) The zebrafish *las1l* gene has three Ensembl transcripts las1l-001, las1l-201 and las1l-202. The *las1l*^{sa674} is a nonsense mutation in exon 11 of transcript las1l-001 converting the codon GAG (Glutamic acid) into TAG (stop codon). The diagrams were taken from Ensembl. Exons are shown as boxes and introns are shown as lines. (B) The human, mouse and zebrafish Las1l proteins are conserved. Boxes show the protein domains and * indicate the location of the *las1l*^{sa674} mutation.

4.2 Results

4.2.1 Gross morphology of *las1l*^{sa674} mutants

The *las1l*^{sa674} mutants, similarly to *nol9*^{sa1022} mutants, appeared normal up to 4 days post fertilisation (d.p.f.). However at 5 d.p.f., they could be distinguished from wild-type siblings under a dissecting microscope (Figure 4-2). The *las1l*^{sa674} mutants had fewer intestinal folds and appeared to have a slightly smaller liver and pancreas compared to wild-type siblings and approximately half of the 5 d.p.f. *las1l*^{sa674} mutants failed to inflate their swim bladder. The morphology of the head, eyes, jaw, size and body shape of *las1l*^{sa674} mutants were indistinguishable from that of wild-type siblings.

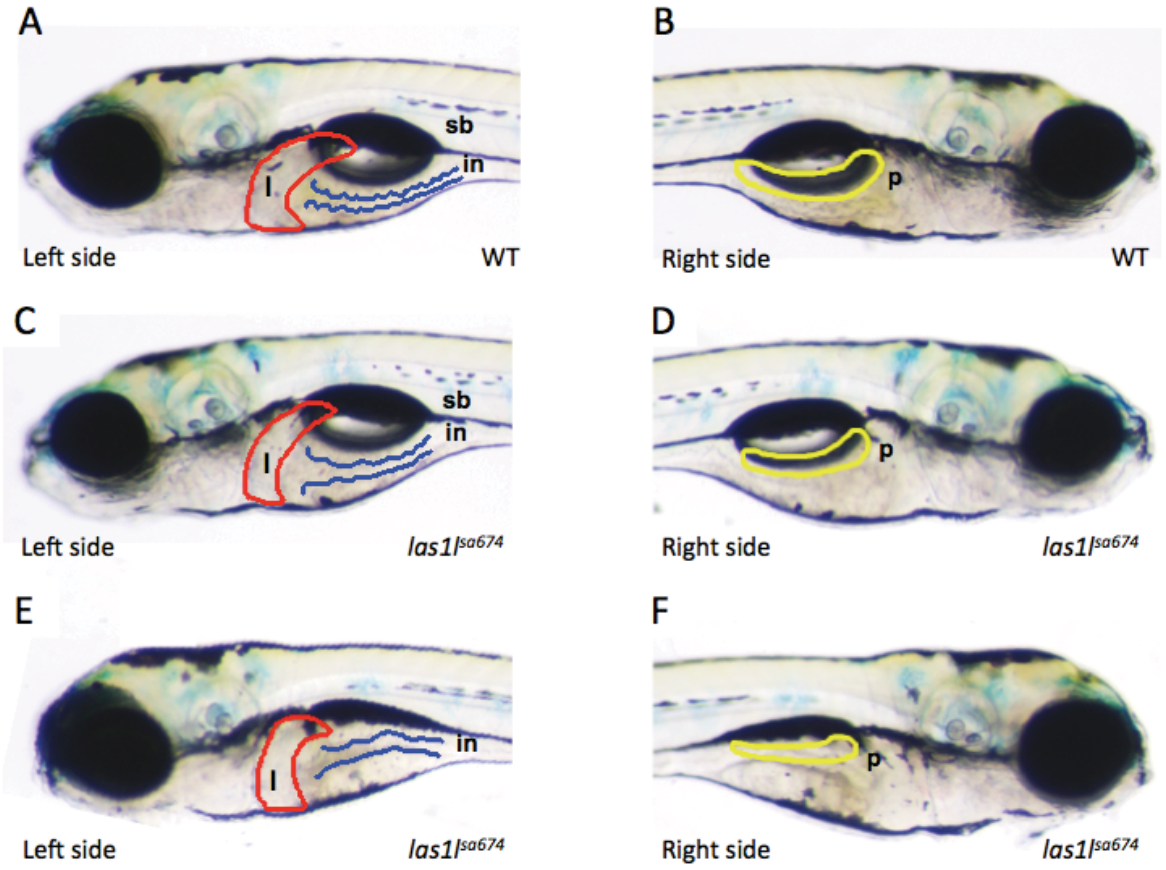

Figure 4-2 The digestive organs of *las1l*^{sa674} mutants are less developed than wild-type siblings. (A-F) Bright-field images of 5 d.p.f. wild-type and *las1l*^{sa674} mutant larvae. (A,C,E) Left side view of wild-type (A) and *las1l*^{sa674} mutants with (C) or without (E) swim bladder showing that the *las1l*^{sa674} mutants have fewer intestinal folds (in; blue) and smaller liver (l; red) compared to wild-type siblings. Swim bladder (sb) is present (C) or absent (E) in *las1l*^{sa674} mutants. (B,D,F) Right side view of wild-type (B) and *las1l*^{sa674} mutants with (D) or without (F) swim bladder showing that *las1l*^{sa674} mutants have a smaller pancreas (p; yellow) compared to wild-type siblings. (WT) wild-type; (l) liver; (in) intestine; (sb) swim bladder; (p) pancreas.

4.2.2 The *las1l*^{sa674} mutants have smaller exocrine pancreas

The pancreas of *las1l*^{sa674} mutants appeared smaller than wild-type siblings under the dissecting microscope. To determine whether *las1l*^{sa674} is causative of the phenotype, offspring from incrosses of F3 *las1l*$^{sa674/+}$ were phenotyped at 5 d.p.f. based on the size of the pancreas under the dissecting microscope and subsequently genotyped (Section 2.1.2). All phenotypic larvae were *las1l*$^{sa674/sa674}$ (n=16) and all non-phenotypic larvae were either *las1l*$^{sa674/+}$ (n=34) or *las1l*$^{+/+}$ (n=16) (Figure 4-3). This data suggests that the *las1l*^{sa674} mutation causes a smaller pancreas and that the phenotype follows a Mendelian pattern of recessive inheritance.

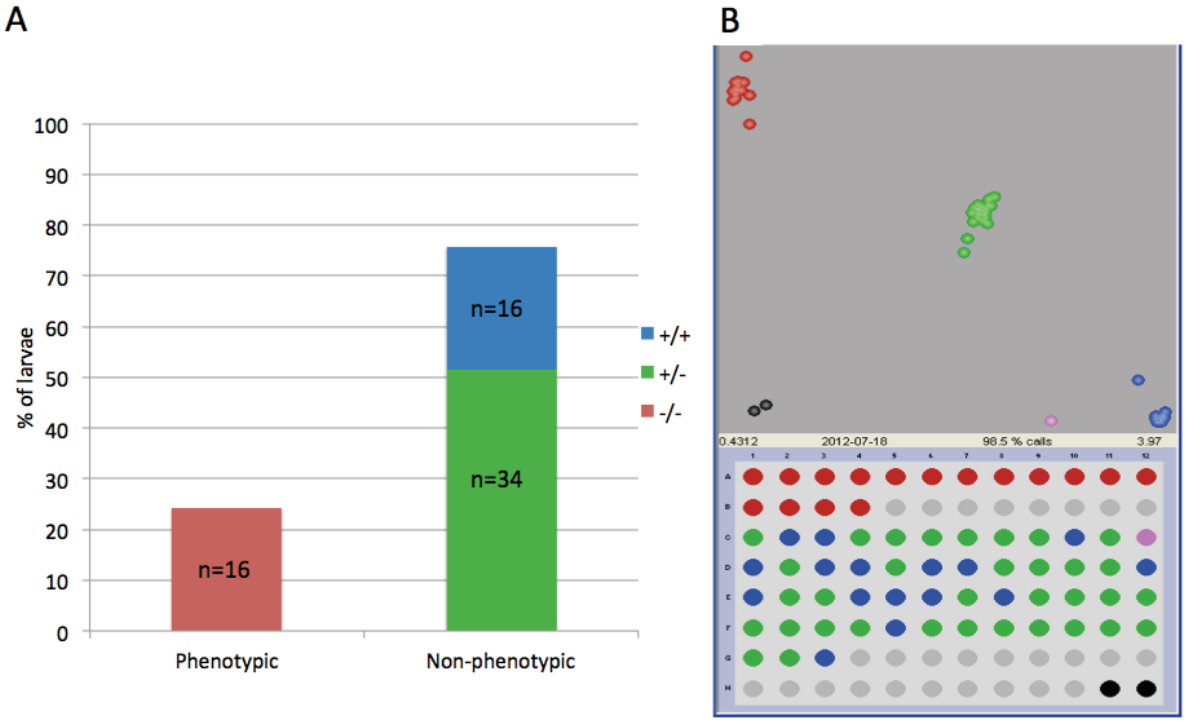

Figure 4-3 The *las1l*^{sa674} mutation is causative of a small pancreas phenotype. (A) Graph showing the percentage of phenotypic and non-phenotypic larvae that are homozygous mutant (-/-; red), heterozygous (+/-; green) and homozygous wild-type (+/+; blue) for *las1l*^{sa674} mutation. (B) KASPar genotyping image. Phenotypic larvae are in the first two rows and non-phenoytpic larvae are in the subsequent five rows. The genotype of homozygous mutant, heterozygous and homozygous wild-type are shown as red, green and blue dots respectively. Pink are uncalled genotypes.

In order to facilitate the study of pancreas development in *las1l*^{sa674} mutants, the F3 *las1l*$^{sa674/+}$ were outcrossed to the transgenic line *Tg(ins:mCherry)*^{jh2}*;Tg(ptf1a:EGFP)*^{jh1} (Pisharath et al., 2007) that express mCherry in the pancreatic islet and EGFP in the exocrine pancreas. Offspring from an incross of *Tg(ins:mCherry)*^{jh2}*;Tg(ptf1a:EGFP)*^{jh1}*;las1l*$^{sa674/+}$ were phenotyped at either 2 d.p.f., 3 d.p.f., 4 d.p.f. or 5 d.p.f. and subsequently genotyped for the *las1l*^{sa674} mutation (Section 2.1.2). At 2 d.p.f., all the embryos had formed an *insulin*-expressing pancreatic islet enclosed by *ptf1a*-expressing tissue. Of the 18 embryos studied, eight were *las1l*^{sa674} mutants (Figure 4-4 A). At 3 d.p.f., the *ptf1a*-expressing region had expanded posteriorly and the *insulin*-positive area had increased in size in all 19 offspring, six of which were *las1l*^{sa674} mutants (Figure 4-4 B). At 4 d.p.f., *las1l*^{sa674} mutants (n=6) could be distinguished from wild-type siblings (n=37) by appearing to have a smaller area of *ptf1a*-expressing cells whilst the *insulin*-positive area appeared similar in size in mutants and wild-type siblings (Figure 4-4 C). At 5 d.p.f., the *ptf1a*-expressing tissue of *las1l*^{sa674} mutants (n=10) appeared smaller than that of wild-type siblings (n=40) whereas the area expressing *insulin* in both mutants and wild-type siblings appeared similar in size (Figure 4-4 D). In both *las1l*^{sa674} mutants and wild-type siblings, the *ptf1a*-positive exocrine tissue had expanded compared to their size at 4 d.p.f. The volume of *ptf1a*-expressing region of *las1l*^{sa674} mutants and wild-type siblings were measured at 5 d.p.f. (Section 2.3.4). The mean volume of *ptf1a*-expressing region of *las1l*^{sa674} mutants (n=10) is statistically significantly smaller than that of wild-type siblings (n=11), 0.00065 mm^3 and 0.002 mm^3 respectively (Student's *t*-test, $p = 1.31 \times 10^{-10}$). These results indicate that the development of the exocrine, but not the *insulin*-positive area, is impaired in *las1l*^{sa674} mutants and that the exocrine pancreas fails to expand normally in *las1l*^{sa674} mutants after 3 d.p.f.

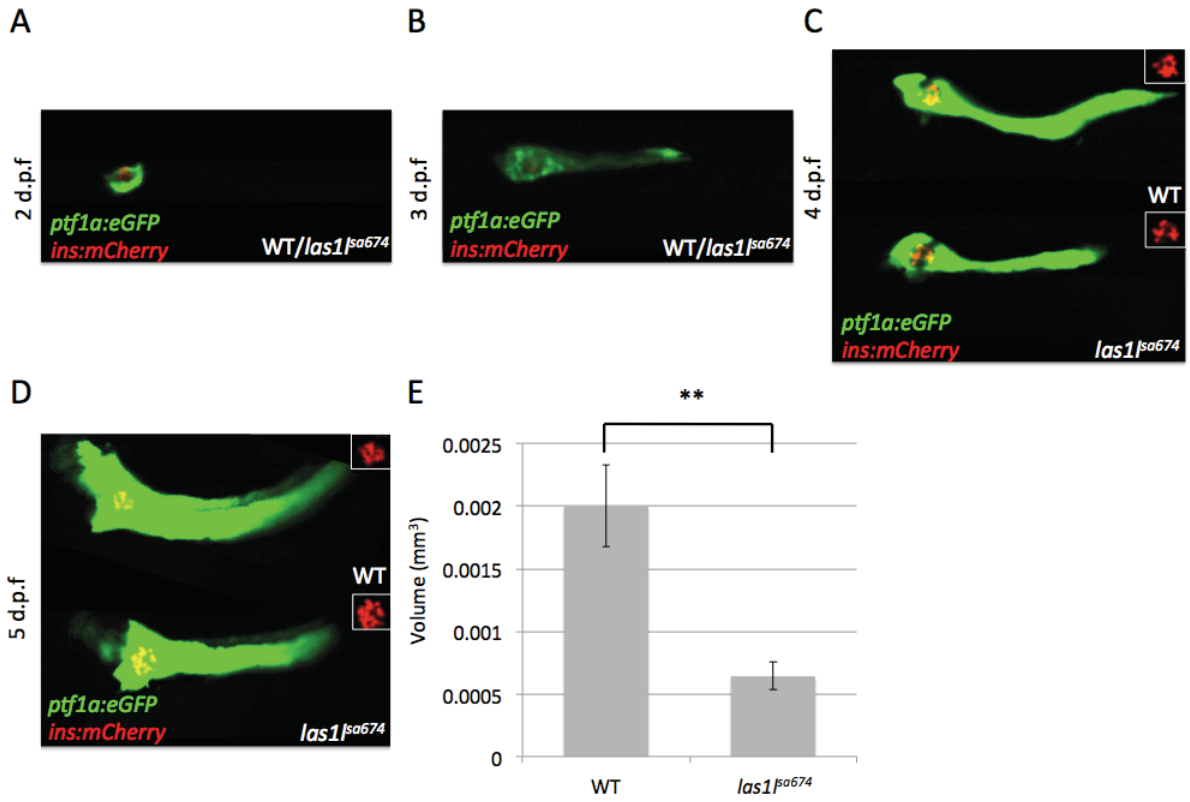

Figure 4-4 Expansion of the exocrine pancreas is impaired in *las1l*^{sa674} mutants. (A-D) Confocal images of 2 d.p.f. to 5 d.p.f offspring from an incross of *Tg(ins:mCherry)*^{jh2}*;Tg(ptf1a:EGFP)*^{jh1}*;las1l*$^{sa674/+}$. (A) Representative image of 2 d.p.f. embryo showing *insulin*-positive pancreatic islet surrounded by *ptf1a*-positive exocrine pancreas. (B) Representative image of 3 d.p.f. embryo showing that the *ptf1a*-positive region has grown. (C-D) At 4 d.p.f. (C) and 5 d.p.f. (D), the *ptf1a*-expressing exocrine pancreas is smaller in *las1l*^{sa674} mutants (upper image) compared to wild-type siblings (lower image) whereas the *insulin*-expressing pancreatic islet (shown in boxed area) is similar in size in *las1l*^{sa674} mutants and wild-type siblings. (E) The volume of *ptf1a*-expressing region of *las1l*^{sa674} mutants (n=10) is significantly smaller than wild-type siblings (n=11). Data is represented as the mean ± SD, Student's *t*-test **p<0.01. (WT) wild-type.

4.2.3 The pancreas of *las1l*^{sa674} mutants do not show increased cell death

The expansion defect of the exocrine pancreas of *las1l*^{sa674} mutants could be explained by increased cell death and/or impaired cell proliferation. The cell death of 4 d.p.f. larvae from an incross of *Tg(ins:mCherry)*^{jh2}*;Tg(ptf1a:EGFP)*^{jh1}*;las1l*$^{sa674/+}$ expressing only EGFP but not mCherry was investigated using TUNEL staining (Figure 4-5) (Section 2.4.5). There were no Tetramethylrhodamine (TMR)-labelled apoptotic cells detected in the pancreas of *las1l*^{sa674} mutants (n=9) or wild-type siblings (n=12). However, the tails of *las1l*^{sa674} mutants and wild-type siblings had a similar mean number of apoptotic cells 5 and 4.6 respectively (Student's *t*-test, $p = 0.50$). These results suggest that the pancreas phenotype of *las1l*^{sa674} mutants does not result from an increase in cell death.

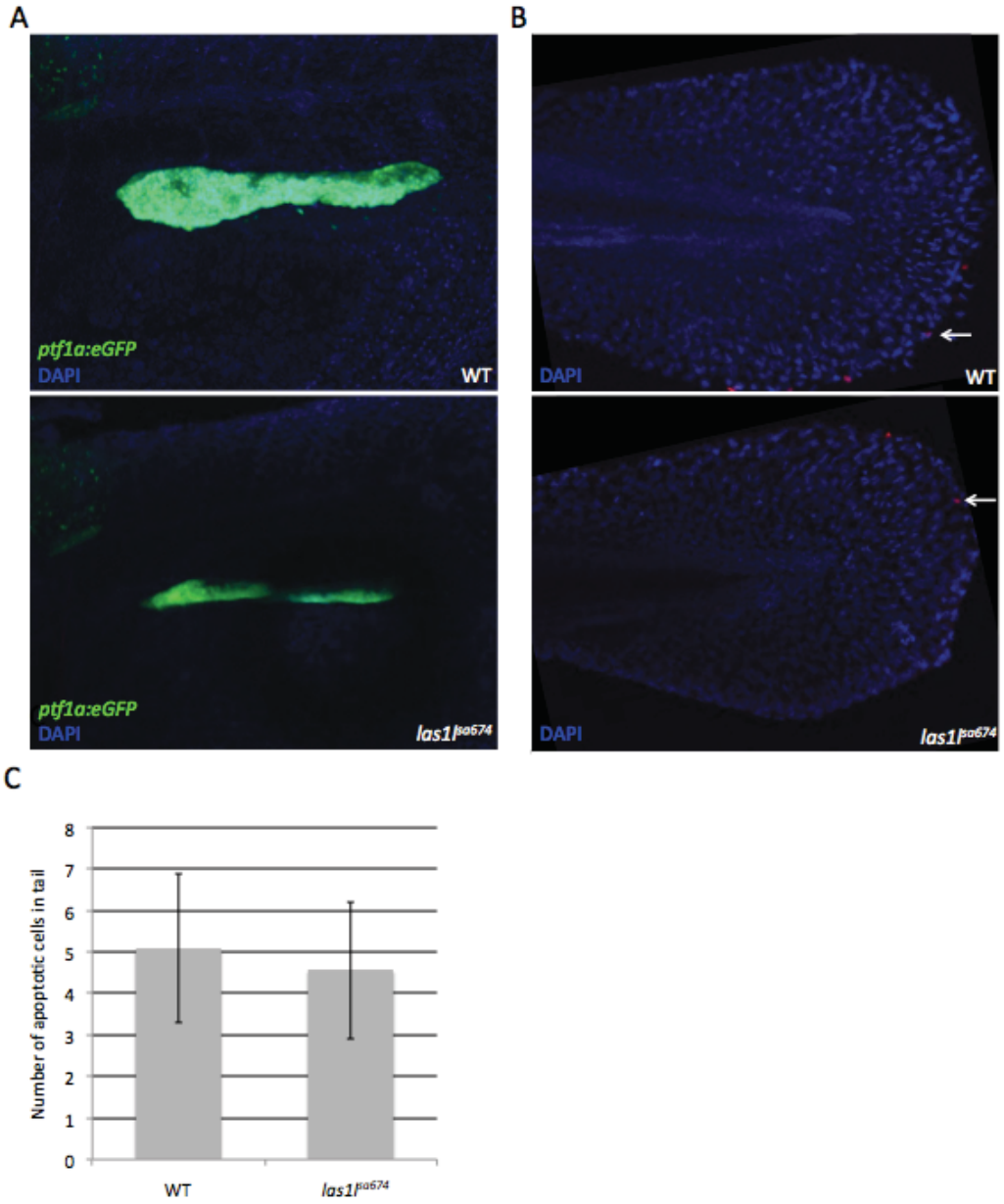

Figure 4-5 The exocrine pancreas of *las1l*^{sa674} mutants do not show increased cell death. (A-B) Maximum intensity projection images of a z series of confocal sections through 4 d.p.f. *las1l*^{sa674} mutant and wild-type zebrafish. Larvae were produced from an incross of *Tg(ins:mCherry)*^{jh2}*;Tg(ptf1a:EGFP)*^{jh1}**;***las1l*$^{sa674/+}$ but expressed only EGFP and subjected to TUNEL and DAPI staining. (A) There are no TMR-labelled apoptotic cells in *ptf1a*-expressing exocrine pancreas of *las1l*^{sa674} mutants and wild-type siblings. (B) Both *las1l*^{sa674} mutants and wild-type siblings have TMR-labelled apoptotic cells (white arrow) in their tails. (C) The number of apoptotic cells in the tails of *las1l*^{sa674} mutants (n=9) and wild-type siblings (n-12) is not statistically significantly different. (WT) wild-type.

4.2.4 Development of the jaw cartilage and erythrocyte is normal in *las1l*^{sa674} mutants

The development of the jaws of *las1l*^{sa674} mutants was investigated since craniofacial defects are common features of ribosomopathies (Narla and Ebert, 2010) and rRNA processing zebrafish mutants displayed jaw defects (Boglev et al., 2013; Chen et al., 2005; Mayer and Fishman, 2003; Qin et al., 2014). At 5 d.p.f., the jaws of *las1l*^{sa674} mutants were indistinguishable to those of wild-type siblings under a dissecting microscope. Alcian blue staining revealed that in *las1l*^{sa674} mutants (n=16) and wild-type siblings (n=43), all the jaw cartilage elements were present, including the Meckel's, palatoquadrate, certohyal and the five ceratobranchial cartilage (Figure 4-6 A) (Section 2.4.6). These results suggest that *las1l*, similarly to *nol9* is not required for jaw development in zebrafish.

The formation of erythrocytes in *las1l*^{sa674} mutants was examined to determine whether *las1l*^{sa674} mutants had erythropoietic defects that are common clinical features of ribosomopathies (Narla and Ebert, 2010). The blood circulation in the heart and tail of all offspring from an incross of *las1l*$^{sa674/+}$ zebrafish was normal at 3 d.p.f. and 5 d.p.f. under the dissecting microscope. O-dianisidine staining revealed that all 3 d.p.f. offspring (n=47) showed a similar area of primitive erythrocytes (Figure 4-6 B) (Section 2.4.7). At 5 d.p.f., haemoglobin-containing erythrocytes were present in both *las1l*^{sa674} mutants (n=16) and wild-type siblings (n=48) (Figure 4-6 C). This data indicates that *las1l*, like *nol9* is not required for the formation of erythrocytes during early development.

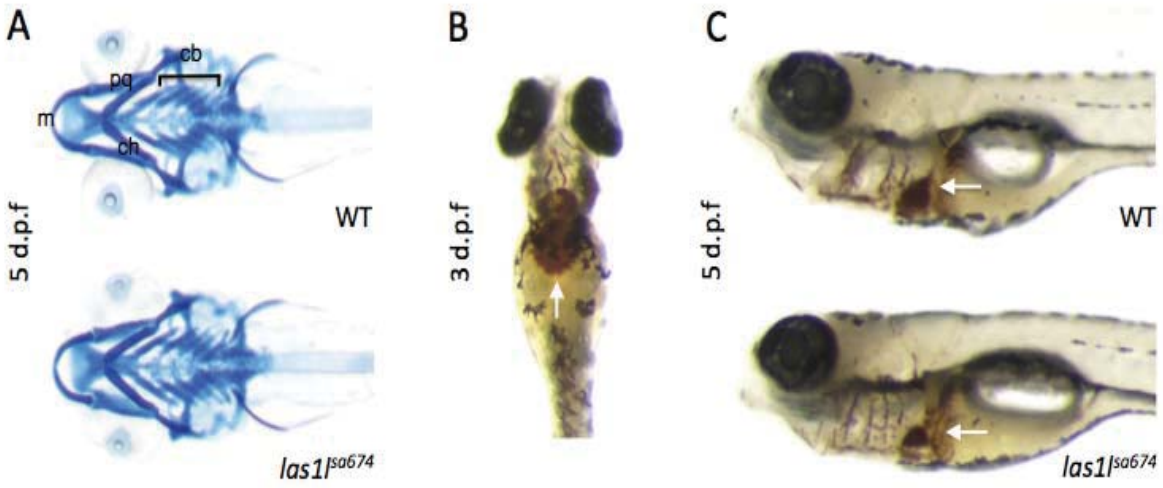

Figure 4-6 Development of the jaw cartilage and erythrocytes are normal in *las1l*^{sa674} mutants. (A) Ventral view with anterior to the left. All the jaw cartilage elements including Meckel's (m), palatoquadrate (pq), ceratohyal (ch) and ceratobranchial cartilage (cb) are present in 5 d.p.f. *las1l*^{sa674} mutants and wild-type siblings as revealed by Alcian blue staining. (B) Ventral view with anterior to the top. Representative image of all 3 d.p.f. embryo from an incross of *las1l*$^{sa654/+}$ stained with o-dianisidine to label haemoglobin-containing erythrocytes (arrow). (C) Left side view of 5 d.p.f. *las1l*^{sa674} mutants and wild-type siblings showing erythrocytes stained with o-dianisidine (arrow). (WT) wild-type; (m) Meckel's; (pq) palatoquadrate; (ch) ceratohyal; (cb) ceratobranchials.

4.3 Discussion

The human LAS1-like (LAS1L) protein is required for cell proliferation and ribosome biogenesis and forms a nucleolar complex with NOL9, PELP1, TEX10, WDR18 and SENP3 (Castle et al., 2012; Castle et al., 2010). All of these proteins are required for the efficient processing of the pre-rRNA internal transcribed spacer-2 (ITS-2) region, maturation of the 28S rRNA and 60S ribosomal subunit synthesis (Castle et al., 2012; Castle et al., 2010). In this project, the *las1l*^{sa674} mutant has been characterised to determine whether Las1l, a Nol9-interacting protein has a similar function to Nol9 during zebrafish development.

The *las1l*^{sa674} mutant displayed underdeveloped digestive organs at 5 d.p.f although the defects appeared to be less severe than those of *nol9*^{sa1022} mutant under the dissecting microscope. Further characterisation of the *las1l*^{sa674} mutant pancreas revealed that the expansion defect of the exocrine pancreas first became apparent between 3 d.p.f. and 4 d.p.f., as was the case for the *nol9*^{sa1022} mutant. At 5 d.p.f., the mean volume of the exocrine pancreas of the *las1l*^{sa674} mutant (0.00065 mm^3) was statistically significantly smaller than that of wild-type siblings (0.0020 mm^3), and nominally larger than that of the *nol9*^{sa1022} mutant (volume=0.0005 mm^3, Student's *t*-test, $p = 0.02$). There was no statistically significant difference in the pancreatic volume of wild-type siblings of *las1l*^{sa674} and *nol9*^{sa1022} mutant (Student's *t*-test, $p = 0.52$). Similarly to *nol9*^{sa1022} mutants, there was no increase in cell death in the pancreas of *las1l*^{sa674} mutants. This suggests that the phenotype is likely to result from impaired cell proliferation as is the case in *nol9*^{sa1022} mutants and this is currently under investigation. The exocrine pancreas of the *las1l*^{sa674} mutant unlike that of the *nol9*^{sa1022} mutant continued to expand after 3 d.p.f. This raises an important question of whether the exocrine pancreas of *las1l*^{sa674} mutant is able to recover from the expansion growth defect.

Recently, a mutation in *LAS1L* has been reported to cause a congenital lethal motor neuron disease (Butterfield et al., 2014). This study however has a major weakness: only one patient was identified as having a mutation in *LAS1L* despite attempts to find additional probands with mutations in the same gene. The pathogenicity of the *LAS1L* mutation is supported by morpholino knockdown of *las1l* in zebrafish that resulted in embryos displaying small malformed brain, shorter necrotic tail, disrupted architecture of peripheral nerve and muscle and early lethality at 24 h.p.f. (Butterfield et al., 2014). The phenotype described in the paper is in contrast with that of the *las1l*^{sa674} mutant where only defects in digestive

organs were observed. There are a number of reasons that could explain this discrepancy. Firstly, *las1l* knockdown was achieved using a translation-blocking morpholino and a splice blocking morpholino that induces skipping of exon 10 whereas the *las1l*^{sa674} mutation is in exon 11. The *las1l* morphants may have less functional Las1l protein than the *las1l*^{sa674} mutants resulting in a more a severe dysmorphic phenotype in the morphants. The expression of Las1l protein in *las1l*^{sa674} mutants is ongoing. Secondly, knocking down *las1l* using morpholinos might have resulted in off-target effects that contribute at least in part to the phenotype described (Robu et al., 2007). Indeed the suppression of the toxic effects of morpholinos by p53 inhibition has not been reported although the phenotype was consistent when using two independent morpholinos and partial rescue was achieved with wild-type but not mutant *LAS1L* RNA (Butterfield et al., 2014). In order to resolve this discrepancy, future work could include generating a zebrafish mutant with the exact mutation from the patient in the equivalent residue in zebrafish via TALENs or CRISPR/Cas technology and examining the axon and muscle development in that mutant.

In general, the phenotype of *las1l*^{sa674} mutant appeared to be less severe than that of *nol9*^{sa1022} mutant. This may be caused by: firstly, the Las1l protein in *las1l*^{sa674} mutant still retaining some functionality particularly since the conserved domain Las1 is encoded by amino acids 12 to 158 and the *lasl*^{sa674} mutation is in amino acid 475. To test this hypothesis, *las1l* function can be disrupted through the generation of a second *las1l* allele with a mutation before or within the Las1 domain using TALENs or CRISPR/Cas technology. Secondly, the requirement of Las1l for expansion of the exocrine pancreas in zebrafish may be less than that of Nol9 although both NOL9 and LAS1L human proteins have been found to be crucial for ribosome biogenesis and cell proliferation (Castle et al., 2012; Castle et al., 2010; Heindl and Martinez, 2010). Thirdly, the maternally deposited supplies of wild-type *nol9* mRNA and/or protein in highly proliferative organs may be exhausted before that of *las1l* mRNA resulting in a more severe phenotype in *nol9*^{sa1022} mutant.

A crucial component of the future work constitutes confirming the role of Las1l in the production of mature 28S rRNA and assembly of 60S ribosomal subunits in zebrafish. The study of *las1l*^{sa674} mutant has not been as detailed as that of *nol9*^{sa1022} mutant due to time constraints. Additional work on the *las1l*^{sa674} mutant should comprise of detailed characterisation of the digestive organs including examining endoderm formation, gut looping, formation of the liver and pancreatic primordia, organ expansion and differentiation

so that comparisons can be drawn between *nol9*^{sa1022} and *las1l*^{sa674} mutant. It would also be interesting to see whether formation of endocrine cells and secondary islets proceed normally as is the case in *nol9*^{sa1022} mutant. These data should help to answer whether Nol9 and Las1l have the same function in zebrafish development. Moreover, the cooperative actions of Nol9 and Las1l proteins can be studied in a *nol9*^{sa1022};*las1l*^{sa674} mutant that we have already generated. We suspect that the combined loss of *nol9* and *las1l* will have an additive effect on digestive organ development. In addition, the developmental expression of *las1l* will be an important aspect to explore. If its expression follows that of *nol9*, *npo*, *def*, *tti*, *nom1* and several *ribosomal protein L* (*rpl*) (Boglev et al., 2013; Chen et al., 2005; Mayer and Fishman, 2003; Provost et al., 2013; Qin et al., 2014), i.e. initially ubiquitously expressed and subsequently preferentially expressed in digestive organs, this will help explain the tissue-specificity of the phenotype observed.

Only the *las1l*^{sa674} mutant was studied since at the time, no other mutants of the other proteins in the complex had been identified. Future directions will include investigating whether Senp3a, Senp3b, Pelp1, Tex10 and Wdr18 have a similar function to Nol9 and Las1l in zebrafish development. At the time of writing, nonsense and/or essential splice site mutations in *senp3b*, *pelp1* and *wdr18* have been identified in ZMP and insertional mutants for *senp3a*, *senp3b* and *wdr18* are also available. These mutants could be studied, complemented with generation of *tex10* mutants to determine whether each of these proteins is involved in expansion growth of digestive organs or development of other organs. In zebrafish, *wdr18* morpholino knock-down experiments showed that Wdr18 is required for the formation of Kupffer's vesicle and for left-right asymmetry (Gao et al., 2011). The morphants displayed a randomised distribution of visceral organs, with the positions of the pancreas and liver either reversed or showing pancreas duplication when the liver was on the opposite position (Gao et al., 2011). Although the expansion growth of the digestive organs has not been studied in the *wdr18* morphants, this study raises the important question of whether proteins of the aforementioned nucleolar protein complex are involved in the regulation of body asymmetry. It is also worth exploring the combined loss of proteins in the complex by incrossing the different mutant lines and transiently knocking down specific genes. This will enhance our knowledge of the role of these proteins in zebrafish development and indicate whether the proteins have synergistic or opposing actions to each other.

Human conditions with mutations in *NOL9*, *SENP3*, *PELP1*, *TEX10* or *WDR18* remain to be identified. Since the function of Nol9 and Las1l have only been described in ribosome biogenesis (Castle et al., 2012; Castle et al., 2010; Heindl and Martinez, 2010) and we have shown that *nol9* and *las1l* zebrafish mutants have comparable digestive-organ specific phenotype, it is possible that mutations in *NOL9* and *LAS1L* in humans will result in a similar disease phenotype. If the congenital lethal motor neuron disease is not actually caused by LAS1L for reasons previously mentioned, we could predict from the zebrafish mutant data that the human condition will likely be characterised by digestive organ failure while craniofacial defects will less likely be present since *las1l*^{sa674} mutant does not have defects in jaw development. Here we found that the blood circulation of *las1l*^{sa674} mutant was normal and that primitive erythrocytes were present at 3 d.p.f. and 5 d.p.f. However, the *las1l*^{sa674} mutant may still have defects in the definitive waves of haematopoiesis comprising of multipotent progenitors of adult cell types. This is currently being explored and therefore it is difficult at present to speculate on the haematopoietic defects if any exist in the human condition. Some of these Las1l-interacting proteins have additional functions to the 28S pre-rRNA processing: PELP1 acts as a transcription coregulator of nuclear hormone receptors (Choi et al., 2004; Vadlamudi et al., 2001) and Wdr18 has an important role in zebrafish development (Gao et al., 2011). Consequently the clinical features resulting from mutations in these proteins will probably not be restricted to digestive organ failure.

The *las1l*^{sa674} mutant, like the *nol9*^{sa1022} mutant, exhibits defects in expansion growth of the exocrine pancreas. This indicates that Las1l and Nol9 have a similar function in zebrafish development and further supports the role of this 28S rRNA processing complex in zebrafish pancreas development.